CLUSTERED MULTI-ANTIGENIC CARBOHYDRATE CONSTRUCTS, METHODS FOR THEIR PREPARATION, AND USES THEREOF

PRIORITY INFORMATION

[0001] This application is a continuation-in-part of co-pending patent application No.: 10/209,618 filed July 31, 2002; which is a continuation-in-part of U.S. patent application No.: 09/641,742 filed August 18, 2000, which claims priority under § 119(e) of the United States Code to provisional application 60/150,088, filed August 20, 1999, entitled "Synthesis and Bioconjugation of the n-Pentenyl Glycoside of the Tumor-Associated Antigen Fucosyl GM1". In addition, the present application claims priority to International Application PCT/US03/22657, filed July 18, 2003. Each of the above applications is hereby incorporated by reference in its entirety.

GOVERNMENT SUPPORT

[0002] The present invention was supported by the National Institutes of Health Grant Numbers: AI16943, AI51883 and CA28824. Therefore, the government has certain rights in the present invention.

BACKGROUND OF THE INVENTION

[0003] The improvement of existing therapeutics and the development of novel therapeutics to treat and/or prolong survival of cancer patients has been the subject of continuing research in the scientific community. Although certain of these efforts have been directed to "traditional" chemotherapeutics (*e.g.*, Paclitaxel and other small molecule and/or natural product based therapies) that act by killing malignant cancer cells, it has also been a long-standing goal (Lanzavechis, *Science*, **260**, 937-944; Pardoll *et al.*, *Curr. Opin. Immunol.* **1993**, *5*, 719-725; Livingston *et al.*, *Curr. Opin. Immunol.* **1992**, *4*, 2; Dranoff *et al.*, *Proc. Natl. Acad. Sci, USA* **1993**, *90*, 3539; M.H. Taoet *et al.*, *Nature*, **1993**, *362*, 755; T. Boon, *Int. J. Cancer* **1993**, *54*, 177) to develop an anti-cancer vaccine that will induce an anticancer immune response. Although cancer vaccines have thus far been perceived as a mode of treatment subsequent to the detection of the disease (for example, by providing an enhanced immunological response), it would be most desirable to develop a selective vaccine that would be able to provide enhanced protection against tumor recurrence and metastasis, for example when the tumor burden has been addressed through surgery, radiation or other chemotherapeutic treatment.

[0004] In general, tumor immunotherapy is based on the theory that tumors possess specific antigens that can be recognized when presented to or processed by a properly trained immune system. The goal for the development of an effective anticancer vaccine is to break the tolerance which the immune system has for these antigens expressed mainly or exclusively by the tumor. One approach researchers have taken has been to present glycoconjugate versions of the antigens, to induce an effective immune response. In an effort to achieve this goal, identified cancer carbohydrate antigens such as TF, Tn, sTN, KH-1, Le^y and Globo-H have been carefully characterized as being over-expressed at the surface of malignant cells in a variety of cancers (breast, colon, prostate, ovarian, liver, small cell lung and adenocarcinomas). In addition, they have been immunocharacterized by monoclonal antibodies and therefore have relevant serological markers available for immunological studies. Such studies have suggested that patients immunized in an adjuvant setting with carbohydrate-based vaccines produce antibodies reactive with human cancer cells, and that the production of such antibodies prohibits tumor recurrence and correlates with a more favorable diagnosis (see, Livingston *et al.*, *J. Cancer Res.* **1989**, *49*, 7045; Ragupathi, G. *Cancer Immunol. Immunother.* **1996**, *43*, 152). Additionally, the isolation and careful structural identification of specific carbohydrate antigens overexpressed in cancer cells has provided a framework for an attack using carbohydrate-based tumor immunotherapy (For reviews see (a) Hakomori, S.; Zhang, Y. *Chem. Biol.* **1997**, *4*, 97; (b) Toyokuni, T.; Singhal, A. K. *Chem. Soc. Rev.* **1995**, *24*, 23 and references therein).

[0005] A major drawback in using carbohydrate epitopes, however, is that they are generally not readily available by isolation from natural sources. For example, the immense difficulties associated with their purification from natural sources render them virtually nonavailable as homogeneous starting materials for a clinical program. Thus, the incorporation of these naturally occurring epitopes into carrier proteins or any favorable molecular context via conjugation for eliciting a therapeutically useful immunological response is inefficient at best, and often virtually impossible. Therefore, to effectively study these vaccines as therapeutic agents, sufficient material can only be obtained by chemical synthesis.

[0006] In an effort to remedy this problem, one of the continuing research efforts is the development of anti-cancer vaccines that incorporate fully synthetic carbohydrate moieties (For a review, see Danishefsky, S.J.; Allen, J.R. *Angew Chem. Int. Ed.* **2000**, *39*, 836-863). One strategy for the development of synthetic anti-cancer vaccines involves the total synthesis of the

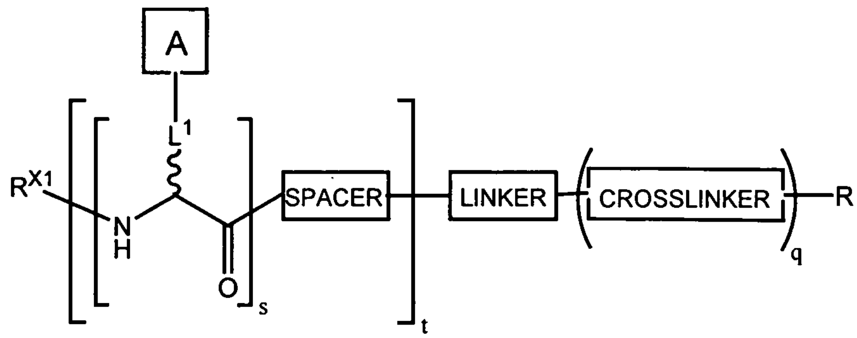
carbohydrate epitope and its subsequent covalent bioconjugation to carrier protein. The vaccine constructs are then subjected to appropriate mouse immunization studies, with the ultimate goal of advancing to human clinical trials. This strategy has resulted in several fully synthetic tumor associated carbohydrate-based vaccines which are at various stages of advanced pre-clinical and clinical processing. In fact, a Globo-H vaccine has undergone clinical evaluation for the treatment of prostate and breast carcinomas at the phase II level (see, for example, Ragupathi *et al.*, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 125) while a Lewis^y antigen-based vaccine, already tested in ovarian cancer, is awaiting more extensive follow-up evaluation (see, Kudryashov *et al. Cancer Immunol. Immunother.* **1998**, *45*, 281).

[0007] Although several synthetic constructs have been developed in recent years, as described above, and in other references described herein, there remains a need for the further investigation to develop novel constructs capable of eliciting a more sustained or effective (and preferably selective) immune response. Clearly, in an effort to achieve this goal, it would be useful to develop improved and/or novel synthetic methods to access heretofore synthetically unavailable antigenic components (*e.g.*, more complex antigenic components such as fucosyl GM1, clustered epitopes and similar structures), or to access non-natural structures [derived from naturally occurring structures] for further immunologic and therapeutic studies.

SUMMARY OF THE INVENTION

[0008] In recognition of the need to further develop novel constructs and improved synthetic methods, the present invention, in one aspect, provides novel glycosides and glycoconjugates glycoamino acids, and methods for the synthesis and use thereof. In another aspect, the present invention provides novel clustered multi-antigenic constructs and methods for the synthesis and use thereof. In yet another aspect, the present invention provides clustered multi-antigenic constructs and novel clustered glycopeptides. In still another aspect, the present invention provides methods for the treatment of cancer, preferably for the prevention of recurrence of cancer, and methods for inducing antibodies in a subject comprising administering to a subject in need, an effective amount of any of the inventive glycoconjugates as disclosed herein.

[0009] In one aspect, the present invention provides novel clustered multi-antigenic constructs having the general structure:



wherein q is 0 or 1;

each occurrence of s is independently an integer from 2-20;

t is an integer from 1-6;

R^{X1} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a nitrogen protecting group, an amino acid or a proctected amino acid;

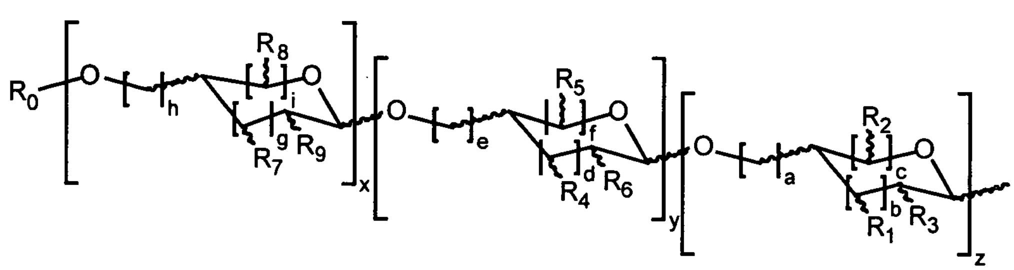
R is hydrogen or an immunogenic carrier;

each occurrence of the spacer is independently a substituted or unsubstituted aliphatic, heteroaliphatic, aryl, heteroaryl or peptidic moiety;

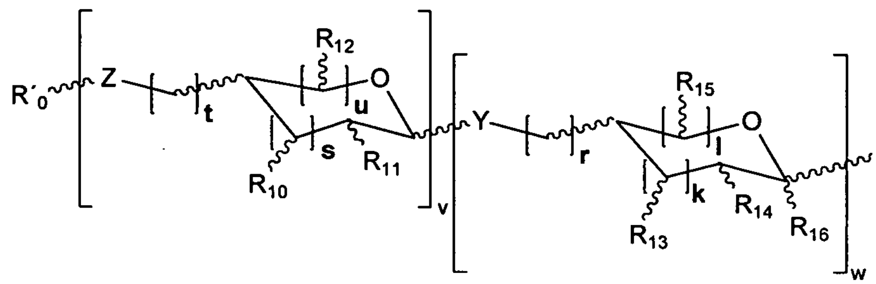
the linker is either a free carboxylic acid, –O-, (carboxamido)alkyl carboxamide, MBS, primary carboxamide, mono- or dialkyl carboxamide, mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester;

each occurrence of L^1 is independently a substituted or unsubstituted aliphatic or heteroaliphatic moiety;

each occurrence of A is independently a carbohydrate domain having the structure:



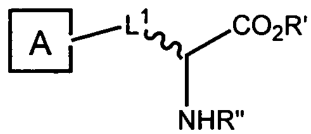
wherein a, b, c, d, e, f, g, h, i, x, y and z are independently 0, 1, 2 or 3, with the proviso that the x, y and z bracketed structures represent furanose or pyranose moieties and the sum of b and c is 1 or 2, the sum of d and f is 1 or 2, and the sum of g and i is 1 or 2, and with the proviso that x, y and z are not simultaneously 0; wherein R_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_1, R_2, R_3, R_4 , R_5, R_6 , R_7, R_8 and R_9 is independently hydrogen, OH, OR^i, NHR^i, $NHCOR^i$, F, CH_2OH, CH_2OR^i, a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i is independently hydrogen, CHO, $COOR^{ii}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group or a saccharide moiety having the structure:



wherein Y and Z are independently NH or O; wherein k, l, r, s, t, u, v and w are each independently 0, 1 or 2; with the proviso that the v and w bracketed structures represent furanose or pyranose moieties and the sum of l and k is 1 or 2, and the sum of s and u is 1 or 2, and with the proviso that v and w are not simultaneously 0; wherein R'_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} is independently hydrogen, OH, OR^{iii}, NHR^{iii}, $NHCOR^{iii}$, F, CH_2OH, CH_2OR^{iii}, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R_{16} is hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, a substituted or unsubstituted linear or branched chain alkyl or aryl group; wherein each occurrence of R^{iii} is hydrogen, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group; and wherein each occurrence of R^{ii} and R^{iv} are each independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; and wherein each glycosidic moiety is either α- or β-linked to an amino acid.

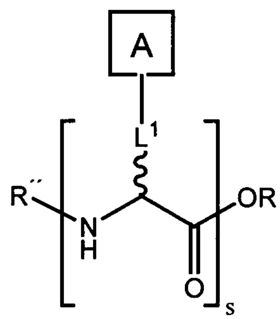
[0010] In another aspect, the present invention provides a method for the synthesis of a clustered multi-antigenic construct, the structure of which is set forth herein, is provided, which comprises steps of:

(a) providing a glycoamino acid having the structure:



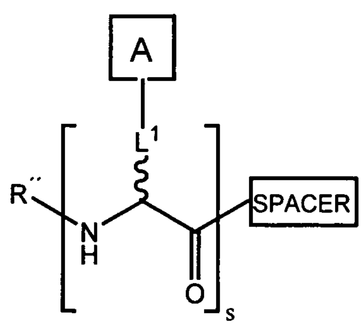
wherein A is a carbohydrate domain as described above;

(b) reacting s occurrences of said glycoamino acid under suitable conditions to generate a glycopeptide having the structure:

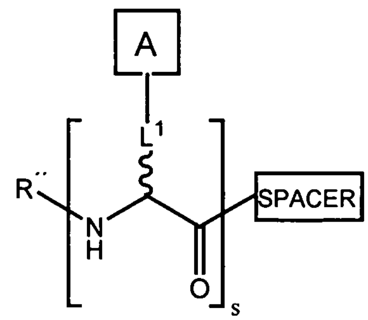


wherein s is an integer from 2-20; each occurrence of A is the same within the bracketed glycopeptide s; R′ is hydrogen or a protecting group; and R′′ is hydrogen, a protecting group, an amino acid or a protected amino acid;

(c) reacting said glycopeptide with a spacer under suitable conditions to generate a spacer construct having the structure:

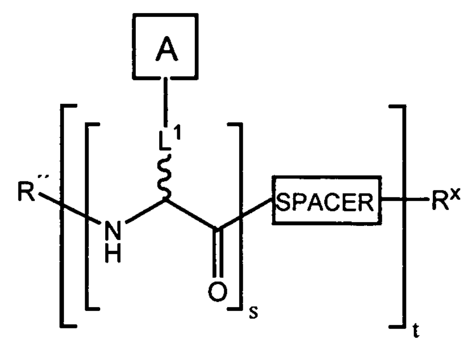


(d) Repeating steps (a) through (c) t-1 times to generate t-1 spacer constructs each independently having the structure:



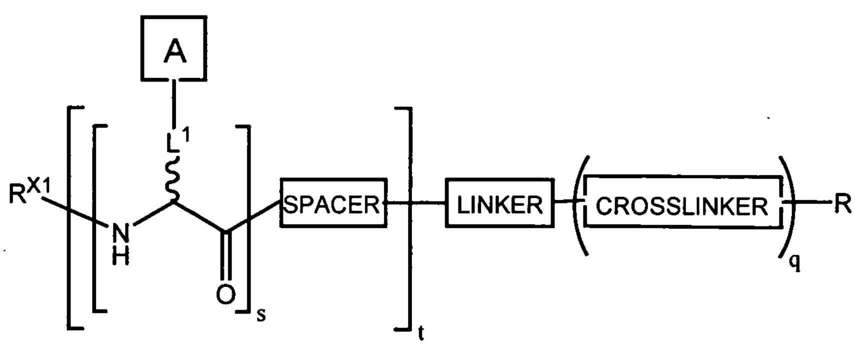
wherein, for each spacer construct, s, L^1, R′′ and the spacer moiety may be the same or different; and each spacer construct comprises a different carbohydrate domain A;

(e) Reacting the spacer construct formed in step (c) with the spacer constructs of step (d) under suitable conditions to generate a construct having the structure:



wherein R^x is a protecting group; each occurrence of A is the same within each bracketed structure s; and each bracketed structure s comprises a different carbohydrate domain A; and

(f) Reacting the constructs of step (e) with a linker and optionally a crosslinker and/or an immunogenic carrier under suitable conditions to form the clustered multi-antigenic construct having the structure:



wherein q, linker, crosslinker, R^{X1} and R are as defined above.

[0011] As detailed herein, in another aspect of the present invention, any of the inventive compounds may be conjugated to generate a glycoconjugate, and may be administered alone,

with an immunogenic carrier (for example, a carrier protein, peptide or lipid), or with an immunological adjuvant or any combination thereof for the treatment of cancer and/or for preventing the recurrence of cancer, or may be administered alone or with an immunological adjuvant to induce antibodies in a subject. In certain exemplary embodiments, when the glycopeptide is a multi-antigenic glycopeptide (*e.g.*, one that comprises at least two different antigenic carbohydrate antigens on the peptide backbone), the antibodies induced recognize the carbohydrate antigens present on the peptidic backbone. In certain embodiments, the carbohydrate antigens present on the peptidic backbone comprise a carbohydrate domain, or truncated or elongated version thereof, that is present on tumor cells.

DEFINITIONS

[0012] Certain compounds of the present invention, and definitions of specific functional groups are described in more detail below. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, the entire contents of which are incorporated herein by reference. Furthermore, it will be appreciated by one of ordinary skill in the art that the synthetic methods, as described herein, utilize a variety of protecting groups. By the term "protecting group", has used herein, it is meant that a particular functional moiety, *e.g.*, O, S, or N, is temporarily blocked so that a reaction can be carried out selectively at another reactive site in a multifunctional compound. In preferred embodiments, a protecting group reacts selectively in good yield to give a protected substrate that is stable to the projected reactions; the protecting group must be selectively removed in good yield by readily available, preferably nontoxic reagents that do not attack the other functional groups; the protecting group may form an easily separable derivative (more preferably without the generation of new stereogenic centers); and the protecting group has a minimum of additional functionality to avoid further sites of reaction. As detailed herein, oxygen, sulfur, nitrogen and carbon protecting groups may be utilized. For example, in certain embodiments, as detailed herein, certain exemplary oxygen protecting groups are utilized. These oxygen protecting groups include, but are not limited to methyl ethers,

substituted methyl ethers (*e.g.*, MOM (methoxymethyl ether), MTM (methylthiomethyl ether), BOM (benzyloxymethyl ether), PMBM (p-methoxybenzyloxymethyl ether), to name a few), substituted ethyl ethers, substituted benzyl ethers, silyl ethers (*e.g.*, TMS (trimethylsilyl ether), TES (triethylsilylether), TIPS (triisopropylsilyl ether), TBDMS (t-butyldimethylsilyl ether), tribenzyl silyl ether, TBDPS (t-butyldiphenyl silyl ether), to name a few), esters (*e.g.*, formate, acetate, benzoate (Bz), trifluoroacetate, dichloroacetate, to name a few), carbonates, cyclic acetals and ketals. In certain other exemplary embodiments, nitrogen protecting groups are utilized. These nitrogen protecting groups include, but are not limited to, carbamates (including methyl, ethyl and substituted ethyl carbamates (*e.g.*, Troc), to name a few) amides, cyclic imide derivatives, N-Alkyl and N-Aryl amines, imine derivatives, and enamine derivatives, to name a few. Certain other exemplary protecting groups are detailed herein, however, it will be appreciated that the present invention is not intended to be limited to these protecting groups; rather, a variety of additional equivalent protecting groups can be readily identified using the above criteria and utilized in the present invention. Additionally, a variety of protecting groups are described in "Protective Groups in Organic Synthesis" Third Ed. Greene, T.W. and Wuts, P.G., Eds., John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference

[0013] It will be appreciated that the compounds, as described herein, may be substituted with any number of substituents or functional moieties. In general, the term "substituted" whether preceded by the term "optionally" or not, and substituents contained in formulas of this invention, refer to the replacement of hydrogen radicals in a given structure with the radical of a specified substituent. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic substituents of organic compounds. For purposes of this invention, heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valencies of the heteroatoms. Furthermore, this invention is not intended to be limited in any manner by the permissible substituents of organic compounds. Combinations of substituents and variables

envisioned by this invention are preferably those that result in the formation of stable compounds useful in the treatment and prevention, for example of disorders, as described generally above. The term "stable", as used herein, preferably refers to compounds which possess stability sufficient to allow manufacture and which maintain the integrity of the compound for a sufficient period of time to be detected and preferably for a sufficient period of time to be useful for the purposes detailed herein.

[0014] The term "aliphatic", as used herein, includes both saturated and unsaturated, straight chain (*i.e.*, unbranched), branched, cyclic, or polycyclic aliphatic hydrocarbons, which are optionally substituted with one or more functional groups. As will be appreciated by one of ordinary skill in the art, "aliphatic" is intended herein to include, but is not limited to, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, and cycloalkynyl moieties. Thus, as used herein, the term "alkyl" includes both straight, branched and cyclic alkyl groups. An analogous convention applies to other generic terms such as "alkenyl", "alkynyl" and the like. Furthermore, as used herein, the terms "alkyl", "alkenyl", "alkynyl" and the like encompass both substituted and unsubstituted groups. In certain embodiments, as used herein, "lower alkyl" is used to indicate those alkyl groups (cyclic, acyclic, substituted, unsubstituted, branched or unbranched) having 1-6 carbon atoms.

[0015] In certain embodiments, the alkyl, alkenyl and alkynyl groups employed in the invention contain 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-4 carbon atoms. Illustrative aliphatic groups thus include, but are not limited to, for example, methyl, ethyl, n-propyl, isopropyl, allyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, sec-pentyl, isopentyl, tert-pentyl, n-hexyl, sec-hexyl, moieties and the like, which again, may bear one or more substituents. Alkenyl groups include, but are not limited to, for example, ethenyl, propenyl, butenyl, 1-methyl-2-buten-l-yl, and the like. Representative alkynyl groups include, but are not limited to, ethynyl, 2-propynyl (propargy1), 1-propynyl and the like.

[0016] The term "alkylamino" refers to a group having the structure -NHR' wherein R' is alkyl, as defined herein. Examples of alkylamino include, but are not limited to, methylamino, ethylamino, iso-propylamino and the like.

[0017] Some examples of substituents of the above-described aliphatic (and other) moieties of compounds of the invention include, but are not limited to: F, Cl, Br, I, OH, NO_2, CN, C(O)-C_1-C_6-alkyl, C(O)-aryl, C(O)-heteroaryl, CO_2-alkyl, CO_2-aryl, CO_2-heteroaryl, $CONH_2$, CONH-C_1-C_6-alkyl, CONH-aryl, CONH-heteroaryl, OC(O)-C_1-C_6-alkyl, OC(O)-aryl, OC(O)-heteroaryl, OCO_2-alkyl, OCO_2-aryl, OCO_2-heteroaryl, $OCONH_2$, OCONH-C_1-C_6-alkyl, OCONH-aryl, OCONH-heteroaryl, NHC(O)-C_1-C_6-alkyl, NHC(O)-aryl, NHC(O)-heteroaryl, $NHCO_2$-alkyl, $NHCO_2$-aryl, NHCONH-heteroaryl, SO_2-C_1-C_6-alkyl, SO_2-aryl, C_3-C_6-cycloalkyl, CF_3, CH_2CF_3, $CHCl_2$, CH_2OH, CH_2CH_2OH, CH_2NH_2, $CH_2SO_2CH_3$, aryl, heteroaryl, benzyl, benzyloxy, aryloxy, heteroaryloxy, C_1-C_6-alkoxy, methoxymethoxy, methoxyethoxy, amino, benzylamino, arylamino, heteroarylamino, C_1-C_3-alkyl-amino, thio, aryl-thio, heteroarylthio, benzyl-thio, C_1-C_6-alkyl-thio, or methylthiomethyl. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples which are described herein.

[0018] In general, the terms "aryl" and "heteroaryl", as used herein, refer to stable mono- or polycyclic, heterocyclic, polycyclic, and polyheterocyclic unsaturated moieties having preferably 3-14 carbon atoms, each of which may be substituted or unsubstituted. Substituents include, but are not limited to, any of the previously mentioned substitutents, *i.e.*, the substituents recited for aliphatic moieties, or for other moieties as disclosed herein, resulting in the formation of a stable compound. In certain embodiments of the present invention, "aryl" refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like. In certain embodiments of the present invention, the term "heteroaryl", as used herein, refers to a cyclic aromatic radical having from five to ten ring atoms of which one ring atom is selected from S, O and N; zero, one or two ring atoms are additional heteroatoms independently selected from S, O and N; and the remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms, such as, for example, pyridyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl,oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, and the like.

[0019] It will be appreciated that aryl and heteroaryl groups (including bicyclic aryl groups) can be unsubstituted or substituted, wherein substitution includes replacement of one, two or three of the hydrogen atoms thereon independently with any one or more of the following moieties including, but not limited to: F, Cl, Br, I, OH, NO_2, CN, C(O)-C_1-C_6-alkyl, C(O)-aryl, C(O)-heteroaryl, CO_2-alkyl, CO_2-aryl, CO_2-heteroaryl, $CONH_2$, CONH-C_1-C_6-alkyl, CONH-aryl, CONH-heteroaryl, OC(O)-C_1-C_6-alkyl, OC(O)-aryl, OC(O)-heteroaryl, OCO_2-alkyl, OCO_2-aryl, OCO_2-heteroaryl, $OCONH_2$, OCONH-C_1-C_6-alkyl, OCONH-aryl, OCONH-heteroaryl, NHC(O)-C_1-C_6-alkyl, NHC(O)-aryl, NHC(O)-heteroaryl, $NHCO_2$-alkyl, $NHCO_2$-aryl, NHCONH-heteroaryl, SO_2-C_1-C_6-alkyl, SO_2-aryl, C_3-C_6-cycloalkyl, CF_3, CH_2CF_3, $CHCl_2$, CH_2OH, CH_2CH_2OH, CH_2NH_2, $CH_2SO_2CH_3$, aryl, heteroaryl, benzyl, benzyloxy, aryloxy, heteroaryloxy, C_1-C_6-alkoxy, methoxymethoxy, methoxyethoxy, amino, benzylamino, arylamino, heteroarylamino, C_1-C_3-alkyl-amino, thio, aryl-thio, heteroarylthio, benzyl-thio, C_1-C_6-alkyl-thio, or methylthiomethyl. Additional examples of generally applicable substitutents are illustrated by the specific embodiments shown in the Examples which are described herein.

[0020] The term "cycloalkyl", as used herein, refers specifically to groups having three to seven, preferably three to ten carbon atoms. Suitable cycloalkyls include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and the like, which, as in the case of other aliphatic, heteroaliphatic or hetercyclic moieties, may optionally be substituted. F, Cl, Br, I, OH, NO_2, CN, C(O)-C_1-C_6-alkyl, C(O)-aryl, C(O)-heteroaryl, CO_2-alkyl, CO_2-aryl, CO_2-heteroaryl, $CONH_2$, CONH-C_1-C_6-alkyl, CONH-aryl, CONH-heteroaryl, OC(O)-C_1-C_6-alkyl, OC(O)-aryl, OC(O)-heteroaryl, OCO_2-alkyl, OCO_2-aryl, OCO_2-heteroaryl, $OCONH_2$, OCONH-C_1-C_6-alkyl, OCONH-aryl, OCONH-heteroaryl, NHC(O)-C_1-C_6-alkyl, NHC(O)-aryl, NHC(O)-heteroaryl, $NHCO_2$-alkyl, $NHCO_2$-aryl, NHCONH-heteroaryl, SO_2-C_1-C_6-alkyl, SO_2-aryl, C_3-C_6-cycloalkyl, CF_3, CH_2CF_3, $CHCl_2$, CH_2OH, CH_2CH_2OH, CH_2NH_2, $CH_2SO_2CH_3$, aryl, heteroaryl, benzyl, benzyloxy, aryloxy, heteroaryloxy, C_1-C_6-alkoxy, methoxymethoxy, methoxyethoxy, amino, benzylamino, arylamino, heteroarylamino, C_1-C_3-alkyl-amino, thio, aryl-thio, heteroarylthio, benzyl-thio, C_1-C_6-alkyl-thio, or methylthiomethyl. Additional examples of generally applicable substitutents are illustrated by the specific embodiments shown in the Examples which are described herein.

[0021] Additionally, it will be appreciated that any of the cycloaliphatic or cycloheteroaliphatic moieties described above and herein may comprise an aryl, heteroaryl, -

(alkyl)aryl or -(alkyl)heteroaryl moiety fused thereto. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0022] As used herein, the terms “aliphatic”, “heteroaliphatic”, “alkyl”, “alkenyl”, “alkynyl”, “heteroalkyl”, “heteroalkenyl”, “heteroalkynyl”, and the like encompass substituted and unsubstituted, saturated and unsaturated, and linear and branched groups. Similarly, the terms “alicyclic”, “heterocyclic”, “heterocycloalkyl”, “heterocycle” and the like encompass substituted and unsubstituted, and saturated and unsaturated groups. Additionally, the terms “cycloalkyl”, “cycloalkenyl”, “cycloalkynyl”, “heterocycloalkyl”, “heterocycloalkenyl”, “heterocycloalkynyl”, “aromatic”, “heteroaromatic”, “aryl”, “heteroaryl” and the like encompass both substituted and unsubstituted groups.

[0023] The term “acyl”, as used herein, refers to a group having the general formula –C(=O)R, where R is an aliphatic, heteroaliphatic, heterocycle, aromatic or heteroaromatic moiety, as defined herein.

[0024] The terms "halo" and "halogen" as used herein refer to an atom selected from fluorine, chlorine, bromine and iodine.

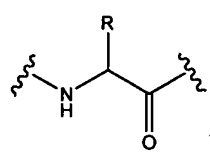
[0025] The phrase, “pharmaceutically acceptable derivative”, as used herein, denotes any pharmaceutically acceptable salt, ester, or salt of such ester, of such compound, or any other adduct or derivative which, upon administration to a patient, provides (directly or indirectly) a compound as otherwise described herein, or a metabolite or residue thereof. Pharmaceutically acceptable derivatives thus include among others pro-drugs. A pro-drug is a derivative of a compound, usually with significantly reduced pharmacological activity, which contains an additional moiety that is susceptible to removal *in vivo* yielding the parent molecule as the pharmacologically active species. An example of a pro-drug is an ester which is cleaved *in vivo* to yield a compound of interest. Pro-drugs of a variety of compounds, and materials and methods for derivatizing the parent compounds to create the pro-drugs, are known and may be adapted to the present invention. Certain exemplary pharmaceutical compositions and pharmaceutically acceptable derivatives will be discussed in more detail herein below.

[0026] The term “treating”, as used herein generally means that the compounds of the invention can be used in humans or animals with at least a tentative diagnosis of disease. The

compounds of the invention will delay or slow the progression of the disease, or will reduce or eliminate one or more symptoms associated with the disease.

[0027] The term "preventing" as used herein means that the compounds of the present invention are useful when administered to a patient who has not been diagnosed as possibly having the disease at the time of administration, but who would be susceptible to developing the disease or be at increased risk for the disease. In certain embodiments, compounds of the invention slow the development of disease symptoms, delay the onset of disease, or prevent the individual from developing the disease at all.

[0028] The term "natural amino acid" as used herein refers to any one of the common, naturally occurring L-amino acids found in naturally occurring proteins: glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), lysine (Lys), arginine (Arg), histidine (His), proline (Pro), serine (Ser), threonine (Thr), phenylalanine (Phe), tyrosine (Tyr), tryptophan (Trp), aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), glutamine (Gln), cysteine (Cys) and methionine (Met).

[0029] The term "unnatural amino acid" as used herein refers to all amino acids which are not natural amino acids. This includes, for example, α-, β-, D-, L- amino acid residues, and compounds of the general formula  wherein the side chain R is other than the amino acid side chains occurring in nature.

[0030] More generally, the term "amino acid", as used herein, encompasses natural amino acids and unnatural amino acids.

BRIEF DESCRIPTION OF THE DRAWING

[0031] Figure 1 depicts Fucosyl GM1, derivatives and constructs thereof.

[0032] Figure 2 depicts synthesis of the ABC trisaccharide **4** and depicts the thioethyl donor **5**.

[0033] Figure 3 depicts the synthesis of hexasaccharide **6a** and the synthesis of Fucosyl GM_1 pentenyl glycoside **1b**. Reagents: (a) MeOTf, CH_2Cl_2:Et_2O (2:1), 0°C, 23%; (b) (i) DMDO, CH_2Cl_2; (ii) PnOH, $ZnCl_2$, -78°C, 65%; (c) TBAF, AcOH, THF; (d) NaOMe, MeOH; (e) NaOH, THF; (f) Na/NH_3, THF –78°C, then MeOH; (g) Ac_2O, pyridine, DMAP, CH_2Cl_2, 46% 5 steps.

[0034] Figure 4 depicts the synthesis of trisaccharide acceptor **15**. Reagents: (a) Ag_2CO_3, cat. I_2, PnOH, CH_2Cl_2, 75%; (b) NaOMe, MeOH; (c) Acetone, cat. PPTS, 44% 2 steps; (d) BnBr, NaH, DMF; 84%; (e) 80% AcOH: H_2O, 90%; (f) **3**, TMSOTf, EtCN, molecular sieves, -40°C, 77%.

[0035] Figure 5 depicts the synthesis of Fucosyl GM_1 Pentenyl Glycoside. Reagents: (a) MeOTf, CH_2Cl_2:Et_2O, 0°C, 70%; (b) TBAF, AcOH, THF; (c) NaOMe, MeOH; (d) NaOH, THF; (e) Na/NH_3, THF, -78°C, then MeOH; (f) Ac_2O, pyridine, DMAP, CH_2Cl_2, 45% 5 steps, (g) steps c-d, 96%.

[0036] Figure 6 depicts the synthesis of Fucosyl GM_1 KLH conjugate **1c**.

[0037] Figure 7 depicts Globo-H, derivatives and constructs thereof.

[0038] Figure 8 depicts a synthetic scheme for a second generation synthesis of globo-H and constructs thereof.

[0039] Figure 9 depicts a retrosynthetic analysis of Globo-H and conjugates thereof.

[0040] Figure 10 depicts the synthesis of glycoside **25** and thioethyl donor **28**. Reagents: (a) HBr, Ac_2O, AcOH, 96%; (b) PentOH, Ag_2CO_3, CH_2Cl_2, 4Å molecular sieves, 75%; c) NaOMe, MeOH; then Dowex-H^+; (d) BnBr, Bu_2SnO, Bu_4NI, C_6H_6, 54% two steps; e) $PhCH(OMe)_2$, CSA, CH_3CN, 72%; (f) BnBr, NaH, DMF, Et_4NI, 97%; (g) $NaCNBH_3$, HCl, Et_2O, THF, 79%; (h) DMDO, CH_2Cl_2; (i) HF/pyridine, 85% two steps; (j) BnBr, NaH, DMF, 95%; (k) $Cp_2Zr(OTf)_2$, toluene/THF 5:1, 80% (α), α:β 10:1; (l) DDQ, CH_3CN, H2O, 84%.

[0041] Figure 11 depicts the synthesis of Globo-H Pentenyl Glycoside (**16c**).

[0042] Figure 12 depicts the conjugation of Globo-H to carrier protein KLH.

[0043] Figure 13 depicts immunoconjugation of tumor antigens Globo-H and Fucosyl GM_1 and the developed glycoamino acid sequence.

[0044] Figure 14 depicts the synthesis of peracetylated lactose amino acid derivatives.

[0045] Figure 15 depicts the synthesis of a peptide containing the Tn antigen, Lewis^y antigen, and the MBr1 antigen. Reagents: (a) TBAF, THF; (b) $AcSCH_2C(O)(CH_2)_3NH_2$, BOP reagent, *i*Pr_2NEt, 54%, 2 steps; (c) TFA, CH_2Cl_2; (d) BOP reagent, *i*Pr_2NEt, 86%, 2 steps; (e) **52**, BOP reagent, *i*Pr_2NEt, 64%, 2 steps; (f) Ac_2O, Et_3N, cat. DMAP, 95%, 2 steps.

[0046] Figure 16 depicts the preparation of fully deprotected glycopeptide **54**.

[0047] Figure 17 depicts the synthesis of α-Tn pentenyl glycoside **40**.

[0048] Figure 18 depicts the synthesis of glycoamino acid **60** using a cross-metathesis reaction.

[0049] Figure 19 depicts exemplary conjugated and non-conjugated trimeric antigenic Gb3 glycopeptides.

[0050] Figures 20 A and 20B depict an exemplary synthesis of trimeric antigenic Gb3 glycopeptides **90** and **92**.

[0051] Figure 21 depicts an exemplary synthesis of trimeric antigenic Gb3 glycopeptides **91** and **93**.

[0052] Figure 22 depicts a pentameric antigenic glycopeptide containing Globo-H, Le^y, STn, TF and Tn antigens.

[0053] Figure 23 depicts the multi-antigenic constructs used in the vaccination studies.

[0054] Figure 24 depicts the monomeric controls used in the vaccination studies.

[0055] Figure 25 depicts FACS histograms corresponding to the analysis of cell surface reactivity of IgM and IgG antibodies in sera from mice immunized with construct **64** against MCF-7.

[0056] Figure 26 depicts an exemplary synthesis of Tn cluster. Reagents and conditions: (a) **107**, HOBt, EDCI, TEA, CH_2Cl_2:DMF, -10 to 23 °C; (b) morpholine, DMF; (c) R-Glycine-OH, HOBt, EDCI, TEA, CH_2Cl_2:DMF, -10 to 23 °C.

[0057] Figure 27 depicts exemplary differential deprotections of Tn cluster. Reagents and conditions: (a) TFA, CH_2Cl_2, (b) morpholine, DMF; (c) 0.1 N NaOH (aq), MeOH

[0058] Figure 28 depicts an exemplary coupling reaction of two protected clusters. Reagents and conditions: (a) HOBt, EDCI, TEA, CH_2Cl_2/DMF, -10 to 23 °C.

[0059] Figure 29 depicts an exemplary coupling reaction of one protected cluster and one deprotected cluster. Reagents and conditions: (a) HOAT, HATU, DIEA, NMP.

[0060] Figure 30 depicts an exemplary coupling reaction of two deprotected clusters. Reagents and conditions: (a) DCC, NMP.

[0061] Figure 31 depicts an exemplary synthesis of TF cluster. Reagents and conditions: (a) **120**, HOBt, EDCI, TEA, CH_2Cl_2:DMF, -10 to 23 °C; (b) morpholine, DMF; (c) Fmoc-Glycine-OH, HOBt, EDCI, TEA, CH_2Cl_2:DMF, -10 to 23 °C.

[0062] Figure 32 depicts an exemplary deprotection of TF cluster. Reagents and conditions: (a) TFA, CH_2Cl_2.

[0063] Figure 33 depicts an exemplary cluster coupling: protected TF and deprotected Tn. Reagents and conditions: (a) HOAT, HATU, DIEA, NMP.

[0064] Figure 34 depicts an exemplary approach for attachment of linker and final deprotection of TF-Tn cluster of clusters.

DETAILED DESCRIPTION OF CERTAIN PREFERRED EMBODIMENTS OF THE INVENTION

[0065] As discussed above, the desire to develop improved methods for the preparation of synthetic vaccines has led to increased research efforts directed toward the synthesis of naturally occurring complex carbohydrate antigens, as well as novel complex structures (*e.g.*, glycopeptides) incorporating these antigenic structures. As is often the case during the course of any such large synthetic undertaking, improved synthetic methods are often developed that can be applied universally. In particular, synthetic studies of naturally occurring antigenic structures have led to the development of novel methodologies enabling the development of heretofore unavailable synthetic carbohydrate-based vaccines. For a review, see Danishefsky, S.J.; Allen, J.R., *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 836-863, and references cited therein.

[0066] Significantly, the present invention provides improved methodologies for the synthesis of complex carbohydrates and related therapeutic compounds (*e.g.*, glycoconjugates and/or glycopeptides). In particular, in the context of synthetic studies developed for the total syntheses of fucosyl GM1 and the n-pentenyl glycoside of Globo-H, generalized methodologies were developed for the improved synthesis of complex carbohydrate structures. This general synthetic method encompasses the realization that the incorporation of an n-alkenyl glycoside protecting group at the reducing end of a carbohydrate acceptor allows for increased coupling efficiencies and accessibility to complex carbohydrates. In yet another aspect, the present invention also provides the recognition that for certain protected carbohydrates, the n-alkenyl moieties can serve as useful precursors that can be utilized ultimately for the synthesis of complex glycopeptides.

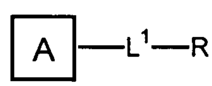
[0067] Furthermore, the present invention also provides the recognition that the presence of the n-alkenyl moiety, whether or not in the context of an antigenic n-pentenyl glycoside or glycopeptide, is advantageous for the development of improved carbohydrate based therapeutics (*e.g.*, synthetic vaccines) because more efficient syntheses of conjugation precursors can be prepared (and ultimately conjugated), and the n-alkenyl carbohydrate also serves as a precursor

for the synthesis of novel n-alkyl glycoamino acids, as described herein. The ability to easily access these glycoamino acids allows for the ultimate synthesis of complex clustered glycopeptides. Significantly, the methodologies provided by the present invention, as described above and in more detail herein, allow the efficient preparation of complex glycopeptide structures having *more than one type* of carbohydrate determinant.

[0068] Specific examples, particularly with respect to the total synthesis of fucosyl GM1 and a novel synthetic scheme for the synthesis of the n-pentenyl glycoside of Globo-H are described in more detail below, along with certain general methodologies developed during the course of these syntheses. It will be appreciated by one of ordinary skill in the art that these examples are not intended to be limiting; rather all equivalents are intended to be incorporated into the scope of the present invention.

[0069] 1) General Description of Compounds of the Invention

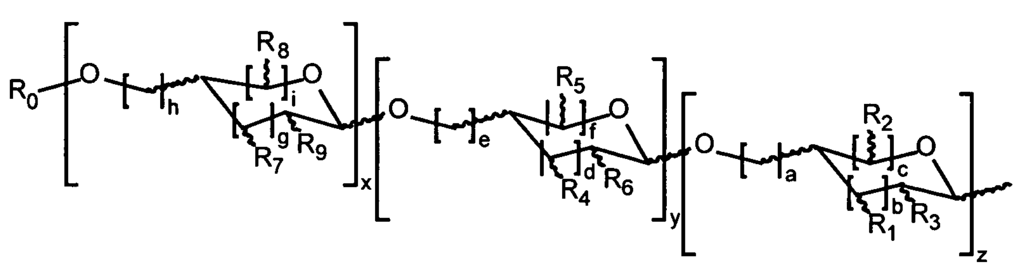
[0070] In one aspect, the present invention provides novel compounds and/or conjugates having the general structure:



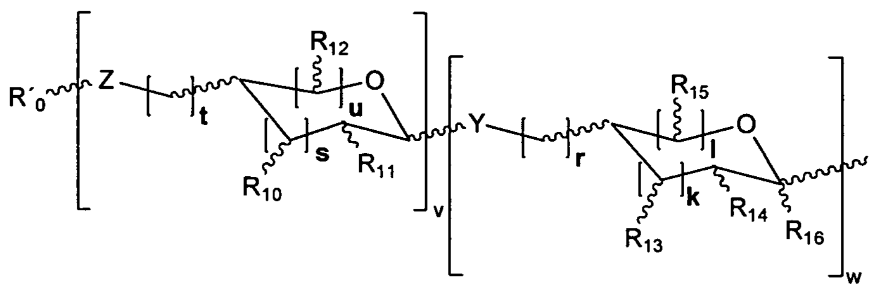
wherein L^1 is a substituted or unsubstituted, linear or branched, cyclic or acyclic, saturated or unsaturated aliphatic or heteroaliphatic moiety;

R is hydrogen; substituted or unsubstituted alkyl; alkenyl; aryl; $-CH_2CH(CO_2R')(NHR'')$, wherein R' or R" are each independently hydrogen, protecting group, substituted or unsubstituted alkyl, a linker, aryl, peptide, protein or lipid; R‴ or NHR‴, wherein R‴ is an immunogenic carrier, peptide, protein or lipid linked to N or the rest of the construct either directly or through a crosslinker;

A is a carbohydrate determinant having the structure:



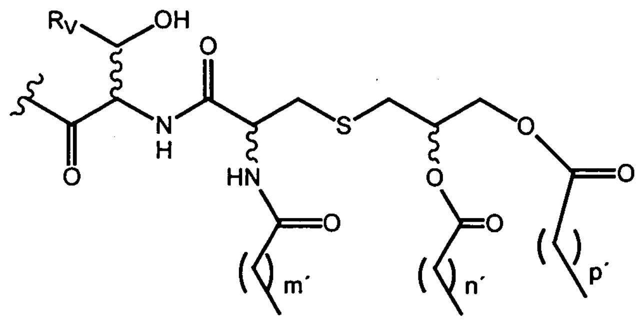
wherein a, b, c, d, e, f, g, h, i, x, y and z are independently 0, 1, 2 or 3, with the proviso that the x, y and z bracketed structures represent furanose or pyranose moieties and the sum of b and c is 1 or 2, the sum of d and f is 1 or 2, and the sum of g and i is 1 or 2, and with the proviso that x, y and z are not simultaneously 0; wherein R_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_1, R_2, R_3, R_4 , R_5, R_6 , R_7, R_8 and R_9 is independently hydrogen, OH, OR^i, NHR^i, $NHCOR^i$, F, CH_2OH, CH_2OR^i, a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i is independently hydrogen, CHO, $COOR^{ii}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group or a saccharide moiety having the structure:



wherein Y and Z are independently NH or O; wherein k, l, r, s, t, u, v and w are each independently 0, 1 or 2; with the proviso that the v and w bracketed structures represent furanose or pyranose moieties and the sum of l and k is 1 or 2, and the sum of s and u is 1 or 2, and with the proviso that v and w are not simultaneously 0; wherein R'_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} is independently hydrogen, OH, OR^{iii}, NHR^{iii}, $NHCOR^{iii}$, F, CH_2OH, CH_2OR^{iii}, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R_{16} is hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, a substituted or unsubstituted linear or branched chain alkyl or aryl group; wherein each occurrence of R^{iii} is hydrogen, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group; and wherein each occurrence of R^{ii} and R^{iv} are each independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group.

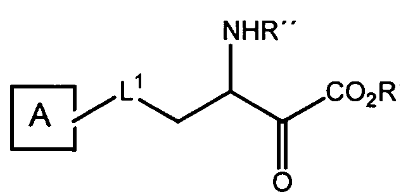
[0071] In certain embodiments, if each occurrence of L^1 is a moiety having the structure $-O(CH_2)_n-$ and if for each occurrence of n, n = 0, then at least one occurrence of A has a different

structure from other occurrences of A. In certain embodiments, each occurrence of L^1 is independently $-O-(CH_2)_n-$, wherein n is 0-9, or a glycoside-containing moiety (*e.g.*, mono saccharide). In certain preferred embodiments, R is allyl, n is 2 and thus the inventive compounds comprise an n-pentenyl moiety. In certain other embodiments, the immunogenic carrier is a protein, peptide, or lipid. In certain other embodiments of the present invention, R is NHR''', and the carrier R''' is KLH or Bovine Serum Albumin. In still other embodiments of the present invention, R is NHR''', and the carrier R''' is a lipid having the structure:



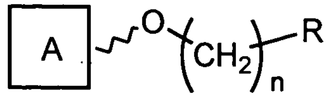
wherein m′, n′ and p′ are each independently integers between about 8 and 20; and R_V is hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl. In certain exemplary embodiments, m′, n′ and p′ are each 14 and the lipid is tripalmitoyl-S-glycerylcysteinylserine (*e.g.*, PamCys). It will be appreciated that the carrier can be linked to the rest of the construct directly or through a crosslinker, and thus R''' incorporates proteins, peptides, and lipids, as well as (crosslinker-protein), (crosslinker-peptide) and (crosslinker-lipid) moieties.

[0065] In other embodiments, the inventive compound is a glycoamino acid and thus R is $CH_2CH(CO_2R')(NHR'')$, which compound has the structure:



[0066] In certain preferred embodiments, the glycoamino acids of the present invention are derived from n-pentenyl glycosides and thus L^1 is $-O(CH_2)_3-$. In certain other preferred embodiments, R' and R'' are each a protecting group independently selected from the group consisting of benzyl, *t*-butyl, TSE (2-(trimethylsilyl)ethyl), Ac (acetyl), Boc (*t*-butoxycarbonyl), Cbz and Fmoc (9-fluoroenyl methoxy carbonyl).

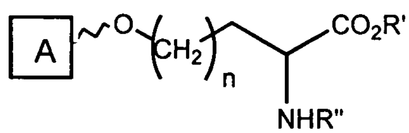
[0067] In certain embodiments, the present invention provides novel compounds and/or conjugates having the general structure:



wherein A and R are as defined generally above and in classes and subclasses herein.

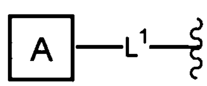
[0068] In certain exemplary embodiments, if A is KH-1, N3, globo-H, glycophorin, Tn, TF, STN, (2,3-ST), 2,6-STn or Le^y, and A is α-O-linked, then n is at least 1. In certain embodiments, A is α- or β-O-linked. In certain preferred embodiments of the present invention, R is allyl, n is 2 and thus the inventive compound is an n-pentenyl moiety.

[0069] In still other embodiments, the inventive compound is a glycoamino acid and thus R is $CH_2CH(CO_2R')(NHR'')$, which compound has the structure:



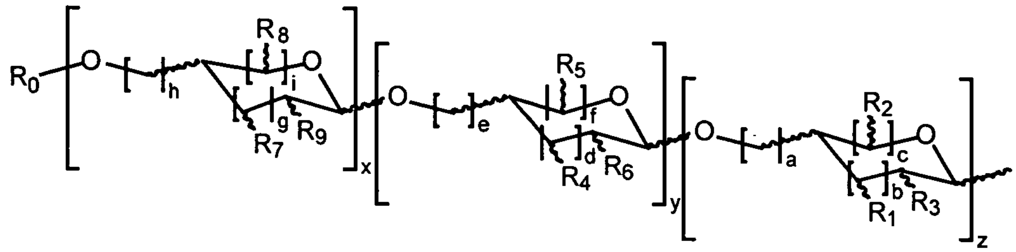
[0070] In certain preferred embodiments, the glycoamino acids of the present invention are derived from n-pentenyl glycosides and thus n is 3. In certain other preferred embodiments, R' and R" are a protecting group, each independently selected from the group consisting of benzyl, t-butyl, TSE, Boc, Fmoc, Cbz and acetyl.

[0071] To date, clustering of α-O-linked antigens has been accomplished with the same antigen across the peptide backbone via the traditional allyl linkage, as described in pending U.S. patent application numbers 09/083,776 and 09/276,595, the entire contents of which are hereby incorporated by reference. However, the present invention efficiently provides peptides having different antigens simultaneously in a clustered format. Thus, in one aspect, the present invention provides multi-antigenic glycopeptides comprising a peptidic backbone made up of two or more amino acids, wherein one or more of said amino acids is/are independently substituted with a glycosidic moiety having the structure:

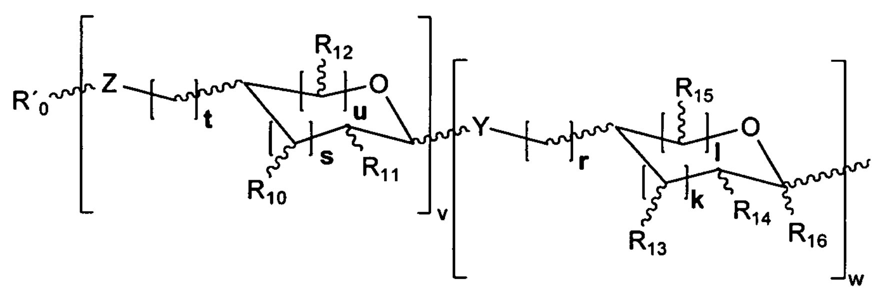


wherein each occurrence of L^1 is independently a substituted or unsubstituted, linear or branched, cyclic or acyclic, saturated or unsaturated aliphatic or heteroaliphatic moiety;

each occurrence of A is independently a carbohydrate determinant having the structure:



wherein a, b, c, d, e, f, g, h, i, x, y and z are independently 0, 1, 2 or 3, with the proviso that the x, y and z bracketed structures represent furanose or pyranose moieties and the sum of b and c is 1 or 2, the sum of d and f is 1 or 2, and the sum of g and i is 1 or 2, and with the proviso that x, y and z are not simultaneously 0; wherein R_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and R_9 is independently hydrogen, OH, OR^i, NHR^i, $NHCOR^i$, F, CH_2OH, CH_2OR^i, a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i is independently hydrogen, CHO, $COOR^{ii}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group or a saccharide moiety having the structure:



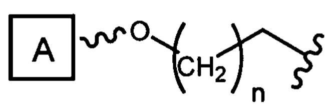
wherein Y and Z are independently NH or O; wherein k, l, r, s, t, u, v and w are each independently 0, 1 or 2; with the proviso that the v and w bracketed structures represent furanose or pyranose moieties and the sum of l and k is 1 or 2, and the sum of s and u is 1 or 2, and with the proviso that v and w are not simultaneously 0; wherein R'_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} is independently hydrogen, OH, OR^{iii}, NHR^{iii}, $NHCOR^{iii}$, F, CH_2OH, CH_2OR^{iii}, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R_{16} is hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, a substituted or unsubstituted linear or branched chain

alkyl or aryl group; wherein each occurrence of R^{iii} is hydrogen, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group; and wherein each occurrence of R^{ii} and R^{iv} are each independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group.

[0072] In certain embodiments, if each occurrence of L^1 is a moiety having the structure –$O(CH_2)_n$- and if for each occurrence of n, n = 0, then at least one occurrence of A has a different structure from other occurrences of A. In certain embodiments, each occurrence of L^1 is independently –O-$(CH_2)_n$-, wherein n is 0-9, or a glycoside-containing moiety (e.g mono- or poly-saccharide).

[0073] It will also be appreciated from the structures as set forth above, that, in addition to providing multi-antigenic structures, the present invention additionally provides clustered structures having one or more saccharide linkages. Thus, in yet another aspect of the present invention, saccharide linked clustered glycopeptides are provided, which glycopeptides may incorporate multiple antigenic structures or may also incorporate all of the same antigenic structures.

[0074] In certain embodiments, L^1 is –O-$(CH_2)_n$-CH_2- and multi-antigenic glycopeptides are provided comprising a peptidic backbone made up of at least three glycoamino acids, wherein one or more of said amino acids is/are substituted with an n-alkyl glycosidic moiety having the structure:



wherein each occurrence of n is independently 0-8; and A is as defined above. In certain embodiments, if for each occurrence of n, n = 0, at least one occurrence of A has a different structure from other occurrences of A. In certain other embodiments, each occurrence of the n-alkyl glycosidic moiety is independently either α- or β-linked to an amino acid residue of the backbone. It will be appreciated that these inventive clustered glycopeptides are not limited to n-alkyl where n is greater than or equal to 1; rather multi-antigenic clustered glycopeptides can be linked via the traditional direct linkage (n=0) or via n-alkyl (such as pentyl) or any combination thereof. In preferred embodiments, each occurrence of A is independently selected from the

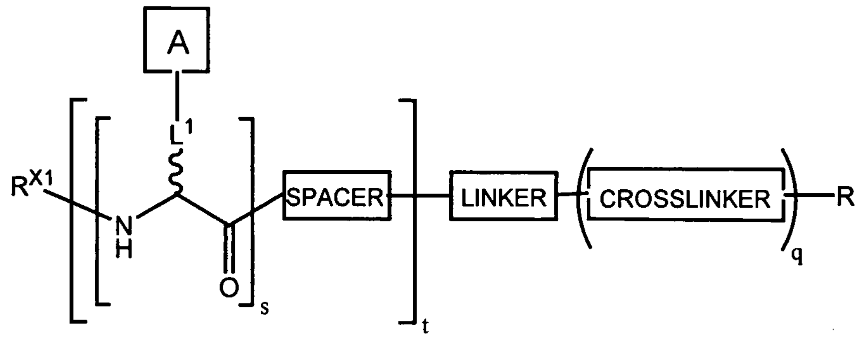
group consisting of Globo-H, fucosyl GM1, KH-1, glycophorin, STN, (2,3)ST, Le^y, Le^x, N3, Tn, 2,6-STn, Gb3 and TF.

[0075] It will also be appreciated from the structures as set forth above, that, in addition to providing multi-antigenic structures, the present invention additionally provides clustered structures having n-alkyl linkages. Thus, in yet another aspect of the present invention, n-alkyl linked (where n is greater than or equal to 1) clustered glycopeptides are provided, which glycopeptides may incorporate multiple antigenic structures or may also incorporate all of the same antigenic structures.

[0076] It will also be appreciated from the structures as set forth above, that the present invention additionally provides clustered structures having n-alkyl linkages or glycoside-containing linkages, or any combination thereof. Thus, in yet another aspect of the present invention, n-alkyl linked (where n is greater than or equal to 1) and/or monosaccharide linked clustered glycopeptides are provided, which glycopeptides may incorporate multiple antigenic structures or may also incorporate all of the same antigenic structures.

[0077] In certain preferred embodiments, the generation of the inventive glycopeptides comprises treating a first glycoamino acid with a deprotecting agent to reveal the corresponding carboxylic acid and then coupling said carboxylic acid under suitable conditions with a spacer moiety and a protecting group to generate a protected amide. A second glycoamino acid can then be coupled under standard conditions (*e.g.*, BOP promoter or other known coupling reagents known in the art of peptide couplings) these couplings can be continued until a peptide of desired length is obtained. It will also be appreciated that solid phase methods of peptide synthesis known in the art can also be employed in the method of the present invention to generate the inventive glycopeptides.

[0078] In certain embodiments, the inventive glycopeptide is a construct having the structure:



wherein q is 0 or 1;

each occurrence of s is independently an integer from 2-20;

t is an integer from 1-6;

R^{X1} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a nitrogen protecting group, an amino acid or a proctected amino acid;

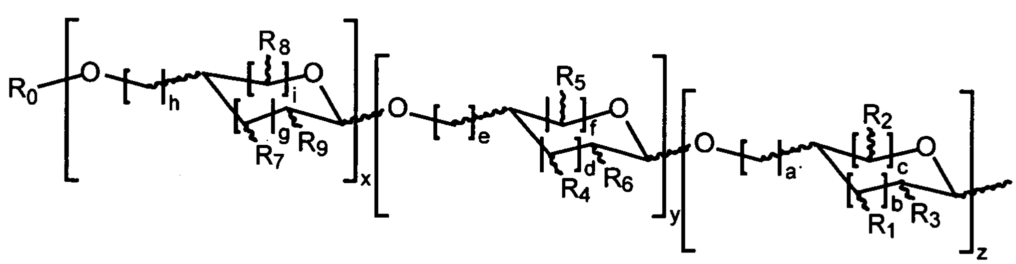
R is hydrogen or an immunogenic carrier;

each occurrence of the spacer is independently a substituted or unsubstituted aliphatic, heteroaliphatic, aryl, heteroaryl or peptidic moiety;

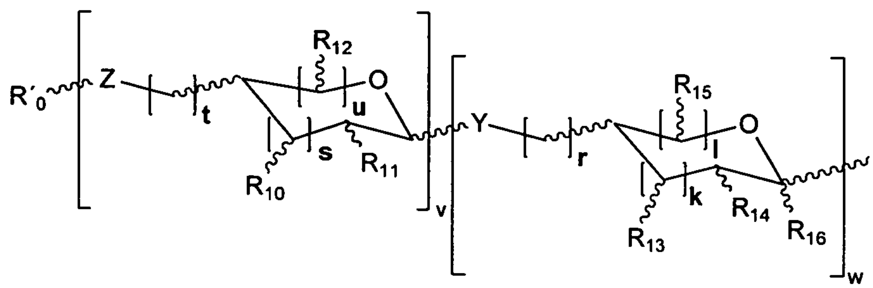
the linker is either a free carboxylic acid, –O-, (carboxamido)alkyl carboxamide, MBS, primary carboxamide, mono- or dialkyl carboxamide, mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester;

each occurrence of L^1 is independently a substituted or unsubstituted aliphatic or heteroaliphatic moiety;

each occurrence of A is independently a carbohydrate domain having the structure:



wherein a, b, c, d, e, f, g, h, i, x, y and z are independently 0, 1, 2 or 3, with the proviso that the x, y and z bracketed structures represent furanose or pyranose moieties and the sum of b and c is 1 or 2, the sum of d and f is 1 or 2, and the sum of g and i is 1 or 2, and with the proviso that x, y and z are not simultaneously 0; wherein R_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_1, R_2, R_3, R_4 , R_5, R_6 , R_7, R_8 and R_9 is independently hydrogen, OH, OR^i, NHR^i, $NHCOR^i$, F, CH_2OH, CH_2OR^i, a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i is independently hydrogen, CHO, $COOR^{ii}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group or a saccharide moiety having the structure:



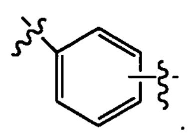
wherein Y and Z are independently NH or O; wherein k, l, r, s, t, u, v and w are each independently 0, 1 or 2; with the proviso that the v and w bracketed structures represent furanose or pyranose moieties and the sum of l and k is 1 or 2, and the sum of s and u is 1 or 2, and with the proviso that v and w are not simultaneously 0; wherein R'_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} is independently hydrogen, OH, OR^{iii}, NHR^{iii}, $NHCOR^{iii}$, F, CH_2OH, CH_2OR^{iii}, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R_{16} is hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, a substituted or unsubstituted linear or branched chain alkyl or aryl group; wherein each occurrence of R^{iii} is hydrogen, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group; and wherein each occurrence of R^{ii} and R^{iv} are each independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; and wherein each glycosidic moiety is either α- or β-linked to an amino acid.

[0079] In certain other embodiments, t is ≥ 2 and within each bracketed structure s, independently, each occurrence of A is the same. In certain embodiments, occurrences of A from one bracketed structure s to the next may be the same or different. In certain embodiments, occurrences of A from one bracketed structure s to the next are different. In certain embodiments, A, for each occurrence, is independently selected from the group consisting of Globo-H, fucosyl GM1, KH-1, glycophorin, N3, Tn, TF, STN, (2,3)ST, 2,6-STn, Gb3, Le^y and Le^x.

[0080] In certain embodiments, L^1 is different for different bracketed structures t, but is the same within each bracketed structure s. In certain embodiments, each occurrence of L^1 is independently a moiety having the structure $-O(CH_2)_n-$ wherein n is an integer from 1-10; or a natural amino acid side chain. In certain embodiments, each occurrence of L^1 is independently a moiety having the structure $-O(CH_2)_n-$ wherein n is an integer from 1-10. In certain other embodiments, each occurrence of L^1 is independently a natural amino acid side chain.

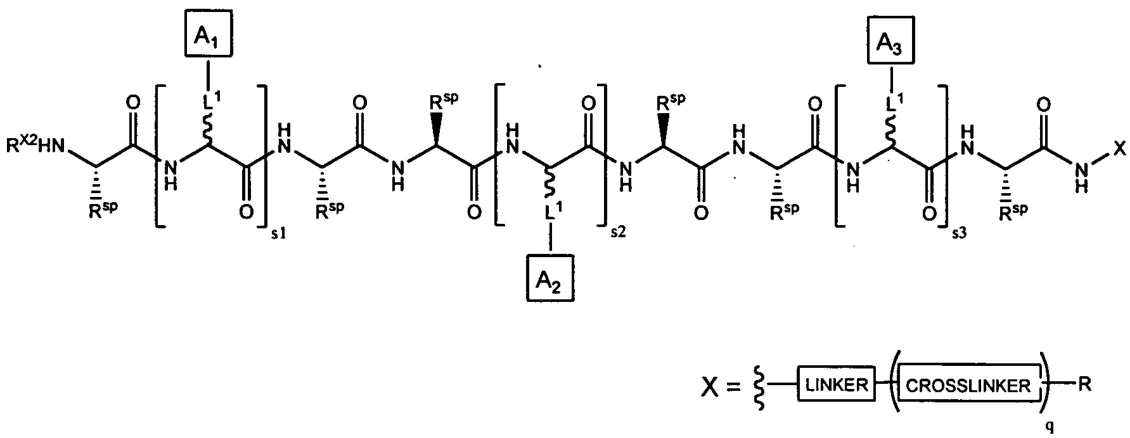
[0081] In certain embodiments, R^{X1} is an acyl moiety. In certain exemplary embodiments, R^{X1} is an amino acid residue.

[0082] In certain embodiments, the spacer, for each occurrence, is independently a substituted or unsubstituted C_{1-6}alkylidene or C_{2-6}alkenylidene chain wherein up to two non-adjacent methylene units are independently optionally replaced by CO, CO_2, COCO, $CONR^{Z1}$, $OCONR^{Z1}$, $NR^{Z1}NR^{Z2}$, $NR^{Z1}NR^{Z2}CO$, $NR^{Z1}CO$, $NR^{Z1}CO_2$, $NR^{Z1}CONR^{Z2}$, SO, SO_2, $NR^{Z1}SO_2$, SO_2NR^{Z1}, $NR^{Z1}SO_2NR^{Z2}$, O, S, or NR^{Z1}; wherein each occurrence of R^{Z1} and R^{Z2} is independently hydrogen, alkyl, heteroalkyl, aryl, heteroaryl or acyl; a peptidyl moiety or a bivalent aryl or heteroaryl moiety. In certain exemplary embodiments, the spacer, for each occurrence, is independently $-(CHR^{sp})_n-$, where n is 1-8 and each occurrence of R^{sp} is independently hydrogen, alkyl, cycloalkyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), -OR^{sp1}, -SR^{sp}1 or $-NR^{sp1}R^{sp2}$ where R^{sp1} and R^{sp1} are independently hydrogen or lower alkyl; a peptidyl moiety comprising one or more α-amino acid residues, or a bivalent aryl moiety having the structure:

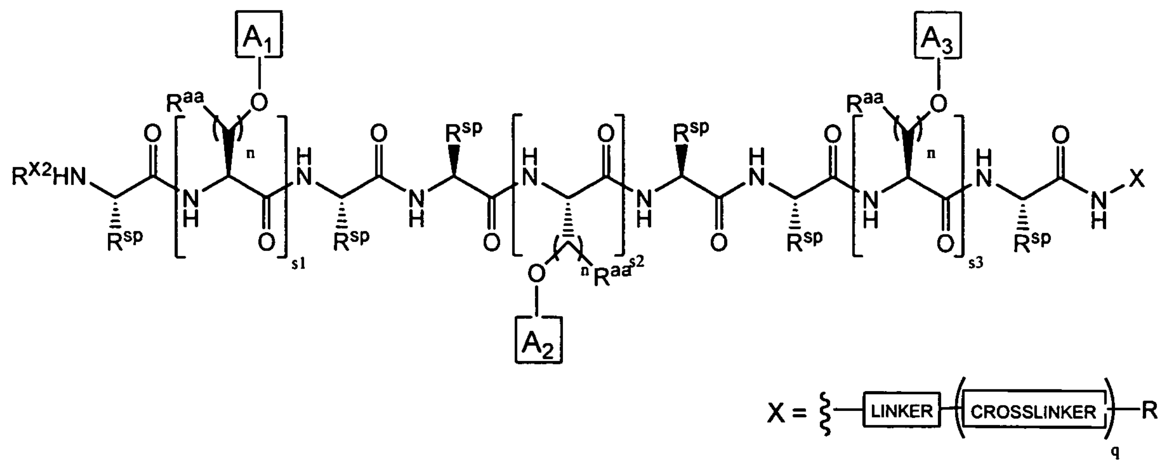


[0083] In certain exemplary embodiments, each occurrence of the spacer is independently a dipeptidyl moiety.

[0084] While the glycopeptides of the present invention is not intended to be limited in size, in certain preferred embodiments, multi-antigenic clusters of clusters having three or more clusters are desirable. In certain exemplary embodiments, t is 3, each occurrence of the spacer that is not directly attached to the linker is independently a dipeptidyl moiety and the glycopeptide has the structure:

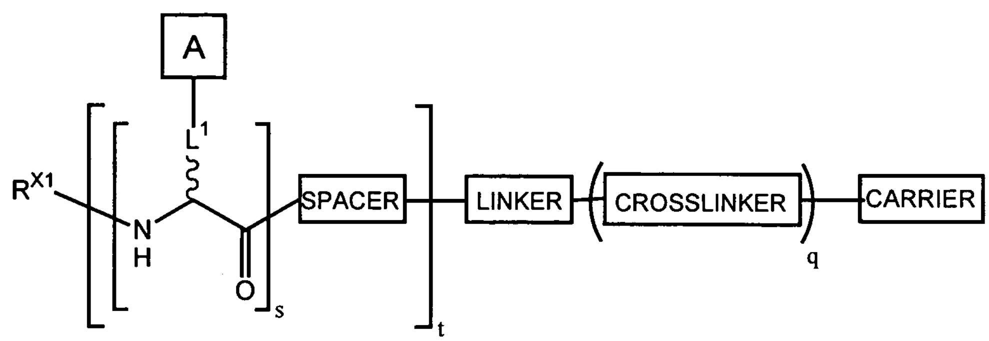


wherein L^1 and R^{sp} are as defined above; s1, s2 and s3 are independently integers from 2-5; A_1-A_3 are carbohydrate domains, as defined above, and are different from each other; and R^{X2} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl) or a nitrogen protecting group. In certain embodiments, L^1 is different for different bracketed structures s1, s2 and s3, but is the same within each bracketed structure s1, s2 and s3. In certain exemplary embodiments, each occurrence of L^1 is independently a natural amino acid side chain. In certain embodiments, each occurrence of L^1 is independently an unnatural amino acid side chain. In certain embodiments, each occurrence of L^1 is independently $-O(CHR^{aa})_n-$ and the glycopeptide has the structure:



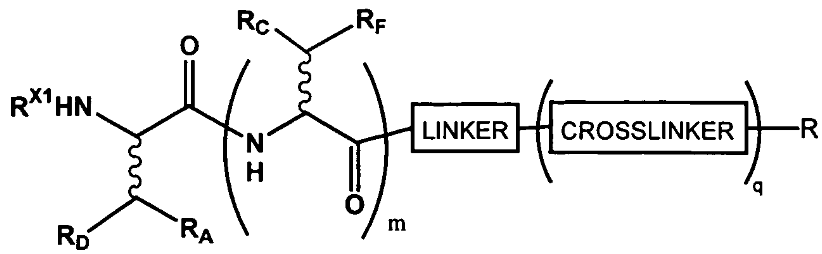
wherein R, R^{X2}, R^{sp}, s1, s2 and s3 and A_1-A_3 are as defined above; each occurrence of n is independently an integer from 1-10; and each occurrence of R^{aa} is hydrogen, lower alkyl, aryl, heteroaryl, -alkyl(aryl) or –alkyl(heteroaryl). In certain exemplary embodiments, each occurrence of n is 1 and each occurrence of R^{aa} is hydrogen or methyl. In certain other exemplary embodiments, each occurrence of n is independently an integer from 1-10 and each occurrence of R^{aa} is hydrogen. In certain embodiments, each occurrence of R^{sp} is independently a natural amino acid side chain. In certain exemplary embodiments, each occurrence of R^{sp} is hydrogen.

[0085] In certain embodiments, the glycopeptide is attached to a suitable immunogenic carrier via a linker and the glycopeptide has the structure:



[0086] In certain embodiments, for the clusters glycopeptides described above and herein, R is a protein, peptide or lipid immunogenic carrier.

[0087] In certain embodiments, the inventive glycopeptide is a construct having the structure:



wherein q is 0 or 1;

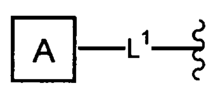
m is an integer from 1-20;

R is hydrogen or an immunogenic carrier;

R^{X1} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a nitrogen protecting group, an amino acid or a proctected amino acid;

the linker is either a free carboxylic acid, –O-, (carboxamido)alkyl carboxamide, MBS, primary carboxamide, mono- or dialkyl carboxamide, mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester;

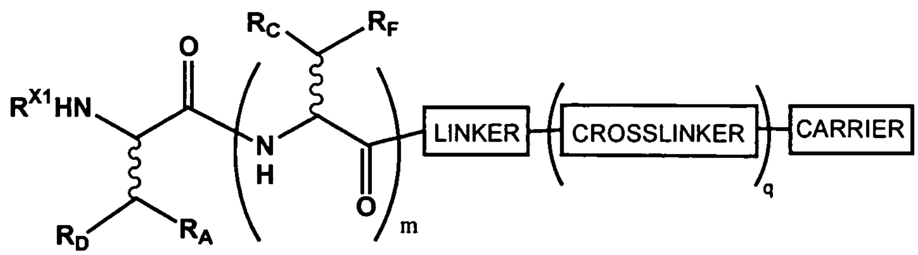
each occurrence of R_A and R_C is independently hydrogen, a subsituted or unsubstituted, linear or branched, cyclic or acyclic, saturated or unsaturated alkyl, aliphatic or heteroaliphatic moiety or a substituted or unsubstituted aryl or heteroaryl moiety; and wherein each occurrence of R_D and R_F is independently a glycosidic moiety having the structure:



wherein A is as defined generally above and in classes and subclasses herein; each occurrence of L^1 is independently a substituted or unsubstituted aliphatic or heteroaliphatic moiety; and wherein each glycosidic moiety A is either α- or β-linked to L^1. In certain embodiments, each occurrence of L^1 is independently a moiety having the structure $-O(CH_2)_n-$ wherein n is an integer from 0-9. In certain embodiments, each occurrence of R_A and R_C is

independently hydrogen or methyl. In still other embodiments, the immunogenic carrier is a protein, peptide or lipid.

[0088] In certain embodiments, the glycopeptide is attached to a suitable immunogenic carrier via a linker and the glycopeptide has the structure:



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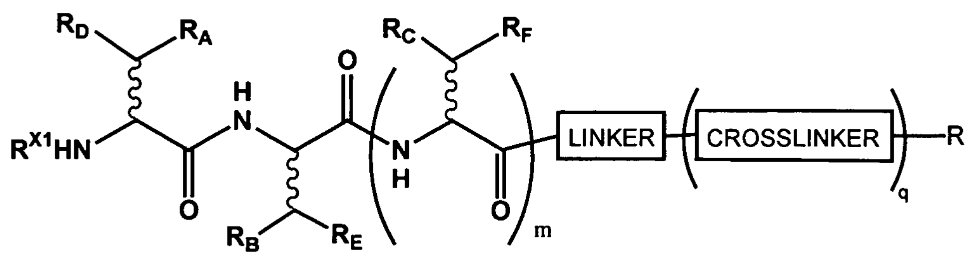
[0089] In certain embodiments, for the above two constructs, m is at least 4. In certain other embodiments, m is at least 5. In yet other embodiments, m is at least 6.

[0090] While the glycopeptide of the present invention is not intended to be limited in size, in certain preferred embodiments, multi-antigenic clusters having three or more antigens are desirable and thus the present invention also provides constructs having the following structure:



wherein q is 0 or 1;

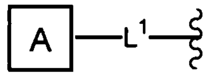
m is an integer from 1-19;

R is hydrogen or an immunogenic carrier;

R^{X1} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a nitrogen protecting group, an amino acid or a proctected amino acid;

the linker is either a free carboxylic acid, -O-, (carboxamido)alkyl carboxamide, MBS, primary carboxamide, mono- or dialkyl carboxamide, mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester;

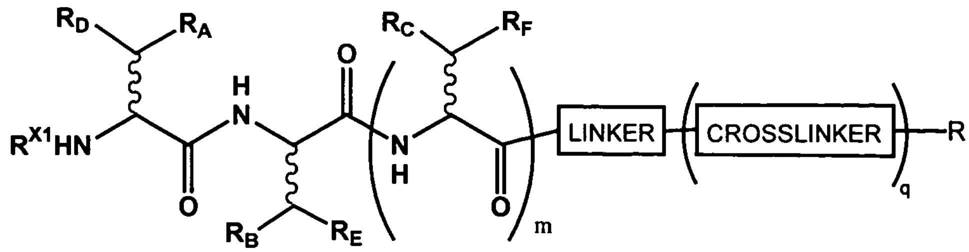
wherein each occurrence of R_A, R_B and R_C is independently hydrogen, a subsituted or unsubstituted, linear or branched, cyclic or acyclic, saturated or unsaturated alkyl, aliphatic or heteroaliphatic moiety or a substituted or unsubstituted aryl or heteroaryl moiety; and wherein each occurrence of R_D, R_E and R_F is independently a glycosidic moiety having the structure:



wherein A is as defined generally above and in classes and subclasses herein; each occurrence of L^1 is independently a substituted or unsubstituted aliphatic or heteroaliphatic moiety.

[0091] In certain embodiments, if each occurrence of L^1 is independently a moiety having the structure $-O(CH_2)_n-$, wherein each occurrence of n is independently 0-9, and if for each occurrence of n, n = 0, then at least one occurrence of A has a different structure from other occurrences of A. In certain other embodiments, the glycosidic moiety is either α- or β-linked to an amino acid residue of the backbone. In certain embodiments, each occurrence of L^1 is independently a moiety having the structure $-O(CH_2)_n-$, wherein each occurrence of n is independently 0-, or a glycoside-containing moiety. In certain exemplary embodiments, at one occurrence of L^1 comprises a monosaccharide. It will be appreciated that these inventive clustered glycopeptides are not limited to n-alkyl where n is greater than or equal to 1; rather multi-antigenic clustered glycopeptides can be linked via the traditional direct linkage (n=0), via n-alkyl (such as pentyl) or via a glycoside moiety or any combination thereof. In other embodiments, each occurrence of A may be the same, however, n-alkenyl (n greater than 1) linkages or glycoside-containing linkages are then utilized. In preferred embodiments, each occurrence of A is independently selected from the group consisting of Globo-H, fucosyl GM1, KH-1, glycophorin, STN, (2,3)ST, Le^y, Le^x, N3, Tn, 2,6-STn, Gb3 and TF. In certain other embodiments, each occurrence of R_A, R_B and R_C is independently hydrogen or methyl. In still other embodiments, the immunogenic carrier is a protein, peptide or lipid.

[0092] In certain embodiments, each occurrence of L^1 is independently a moiety having the structure $-O(CH_2)_n-$ and the multi-antigenic glycopeptide has the following structure:



wherein q is 0 or 1;

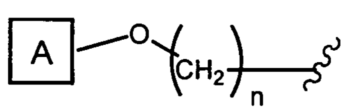
m is an integer from 1-19;

R is hydrogen or an immunogenic carrier;

R^{X1} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a nitrogen protecting group, an amino acid or a proctected amino acid;

the linker is either a free carboxylic acid, -O-, (carboxamido)alkyl carboxamide, MBS, primary carboxamide, mono- or dialkyl carboxamide, mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester;

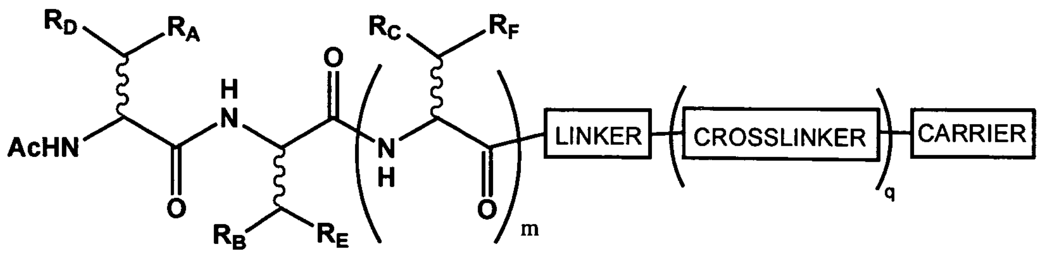
each occurrence of R_A, R_B and R_C is independently hydrogen, a substituted or unsubstituted, linear or branched, cyclic or acyclic, saturated or unsaturated aliphatic or heteroaliphatic moiety or a substituted or unsubstituted aryl or heteroaryl moiety; and wherein each occurrence of R_D, R_E and R_F is independently an alkyl glycosidic moiety having the structure:



wherein each occurrence of A is independently a carbohydrate domain as defined generally above and in classes and subclasses herein; and wherein each occurrence of n is independently 0-8. In certain embodiments, if for each occurrence of n, n = 0, at least one occurrence of A has a different structure from other occurrences of A. In certain other embodiments, each occurrence of A is independently either α- or β-linked to an amino acid

residue of the backbone via the $-O(CH_2)_n-$ linker. In certain embodiments, each occurrence of R_A, R_B and R_C is independently hydrogen or methyl. In still other embodiments, the immunogenic carrier is a protein, peptide or lipid.

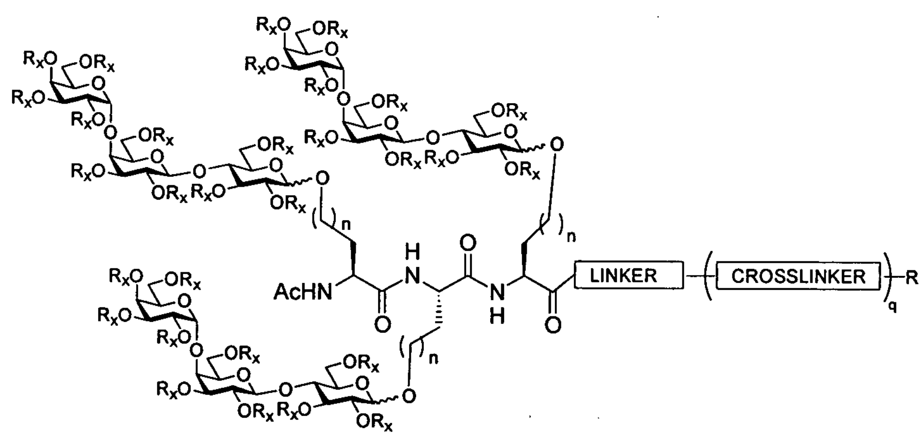
[0093] In certain embodiments, the glycopeptide is attached to a suitable immunogenic carrier via a linker and the glycopeptide has the following structure:



wherein the carrier is an immunogenic carrier;

[0094] In certain embodiments, for the above three constructs, m is at least 3. In certain other embodiments, m is at least 4. In yet other embodiments, m is at least 5. In yet other embodiments, m is at least 6.

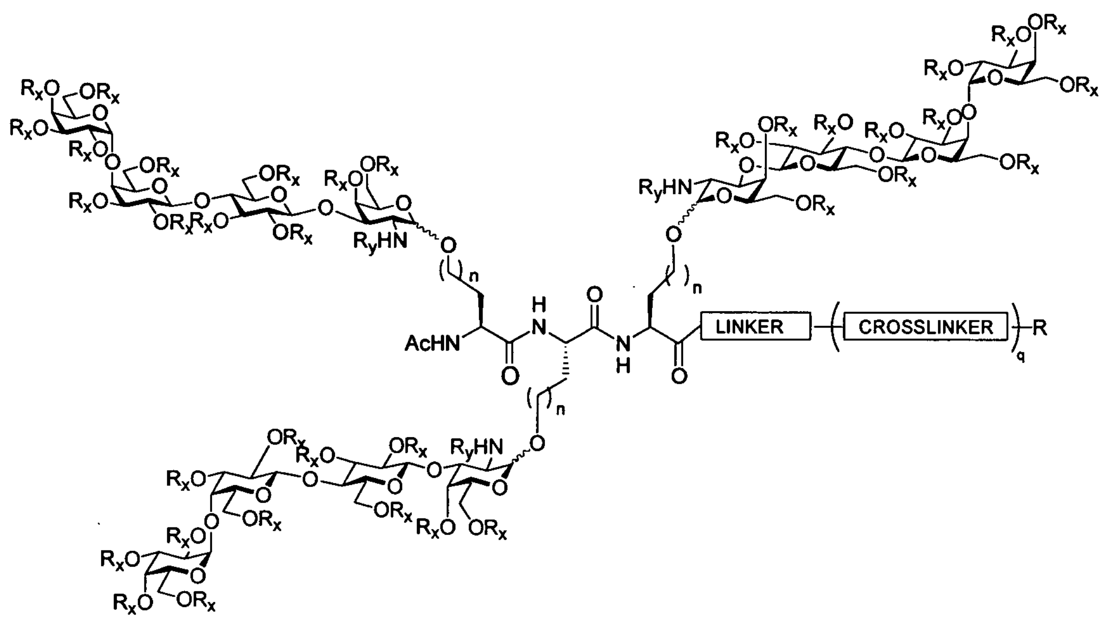
[0095] In certain exemplary embodiments, the present invention provides a novel trimeric antigenic glycopeptide incorporating three occurrences of Gb3, to generate a novel trimeric antigenic compound having the structure:



wherein each occurrence of R_x is independently hydrogen or a protecting group; q is 0 or 1; each occurrence of n is independently an integer from 0-9; R is hydrogen or an immunogenic carrier. In certain exemplary embodiments, the immunogenic carrier is a protein, peptide or lipid. In certain embodiments, each occurrence of n is 3. In certain other embodiments, q is 0. In still

other embodiments, the immunogenic carrier is KLH, Human Serum Albumin, Bovine Serum Albumin or polylysine. In certain other embodiments, the immunogenic carrier is PamCys. In yet other embodiments, the linker is a moiety having the structure $-NH(CH_2)_tNHC(=O)(CH_2)_vS-$ wherein t and v are each integers from 1-6. In certain embodiments, t is 3 and v is 1. In certain exemplary embodiments, each occurrence of R_x is hydrogen. In certain exemplary embodiments, n and q are each 0, the immunogenic carrier is KLH or PamCys and the linker is a moiety having the structure $-NH(CH_2)_tNHC(=O)(CH_2)_vS-$ wherein t and v are each integers from 1-6. In certain embodiments, one or more of the carbohydrate domains is/are α-linked to the peptide backbone.

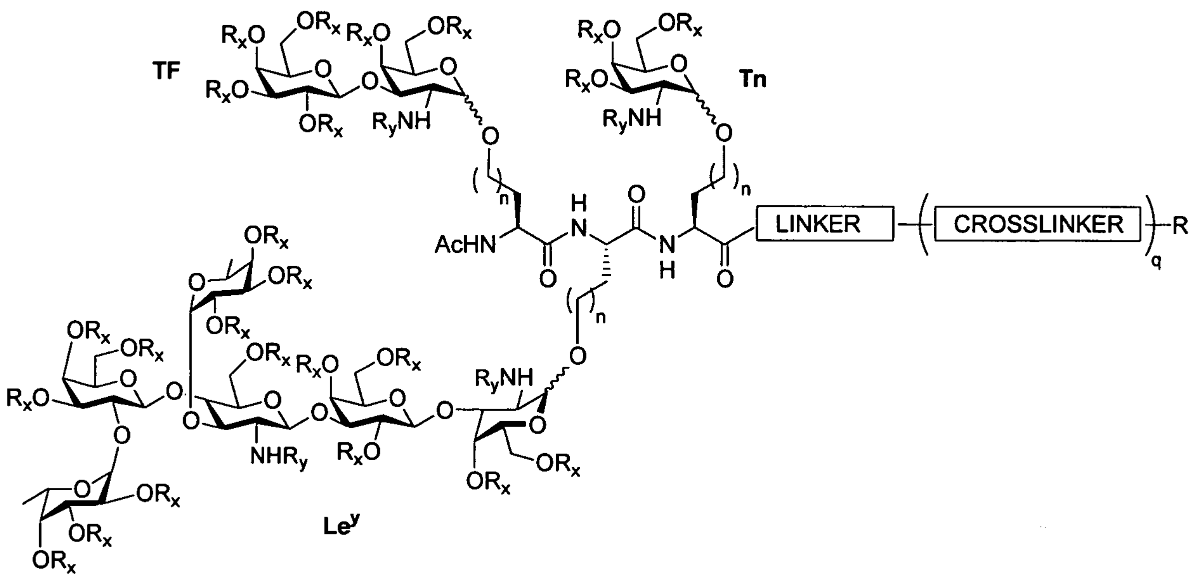
[0096] In certain exemplary embodiments, the present invention provides a novel trimeric antigenic glycopeptide incorporating three occurrences of Gb3, to generate a novel trimeric antigenic compound having the structure:



wherein each occurrence of R_x and R_y is independently hydrogen or a protecting group; q is 0 or 1; each occurrence of n is independently an integer from 0-9; R is hydrogen or an immunogenic carrier. In certain exemplary embodiments, the immunogenic carrier is a protein, peptide or lipid. In certain embodiments, each occurrence of n is 0. In certain other embodiments, q is 0. In still other embodiments, the immunogenic carrier is KLH, Human Serum Albumin, Bovine Serum Albumin or polylysine. In certain other embodiments, the immunogenic carrier is PamCys. In yet other embodiments, the linker is a moiety having the

structure –NH(CH2)$_t$NHC(=O)(CH2)$_v$S- wherein t and v are each integers from 1-6. In certain exemplary embodiments, each occurrence of R_x is hydrogen and each occurrence of R_y is Ac. In certain exemplary embodiments, n and q are each 0, the immunogenic carrier is KLH or PamCys and the linker is a moiety having the structure $-NH(CH_2)_tNHC(=O)(CH_2)_vS-$ wherein t and v are each integers from 1-6. In certain embodiments, one or more of the carbohydrate domains is/are α-linked to the peptide backbone.

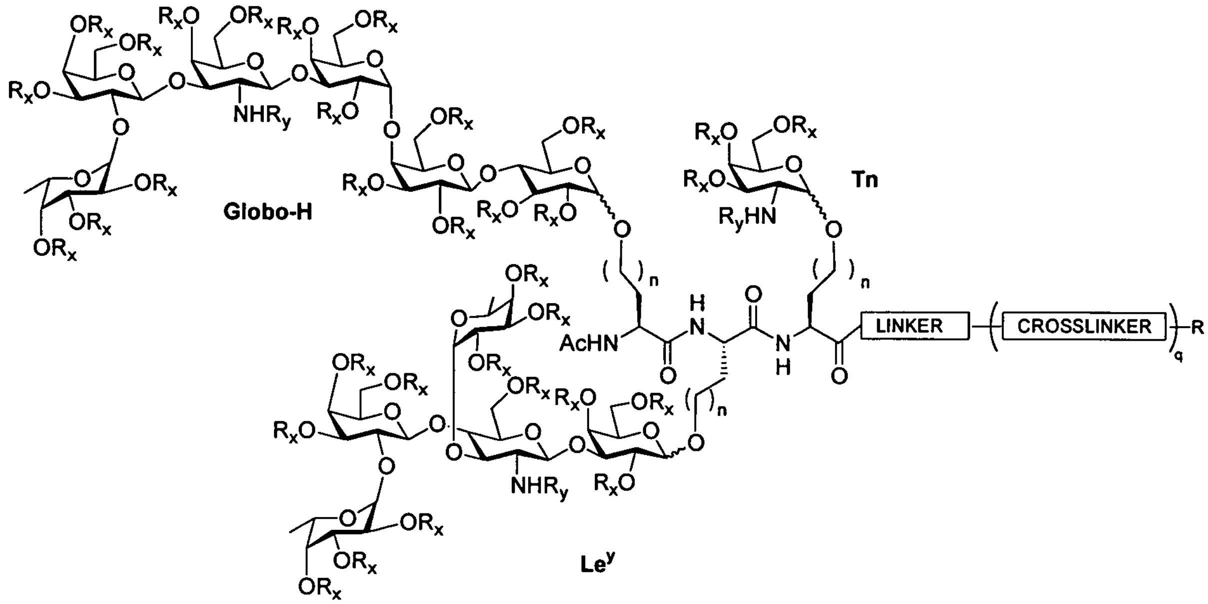
[0097] In certain exemplary embodiments, the present invention provides a novel trimeric antigenic glycopeptide incorporating TF, Le^y and Tn, to generate a novel trimeric antigenic compound having the structure:



wherein each occurrence of R_x and R_y is independently hydrogen or a protecting group; q is 0 or 1; each occurrence of n is independently an integer from 0-9; R is hydrogen or an immunogenic carrier. In certain exemplary embodiments, the immunogenic carrier is a protein, peptide or lipid. In certain embodiments, each occurrence of n is 0. In certain other embodiments, q is 0. In still other embodiments, the immunogenic carrier is KLH, Human Serum Albumin, Bovine Serum Albumin or polylysine. In certain other embodiments, the immunogenic carrier is PamCys. In yet other embodiments, the linker is a moiety having the structure $-NH(CH_2)_tNHC(=O)(CH_2)_vS-$ wherein t and v are each integers from 1-6. In certain embodiments, t is 3 and v is 1. In certain exemplary embodiments, each occurrence of R_x is hydrogen and each occurrence of R_y is Ac. In certain exemplary embodiments, n and q are each 0, the immunogenic carrier is KLH or PamCys and the linker is a moiety having the structure –

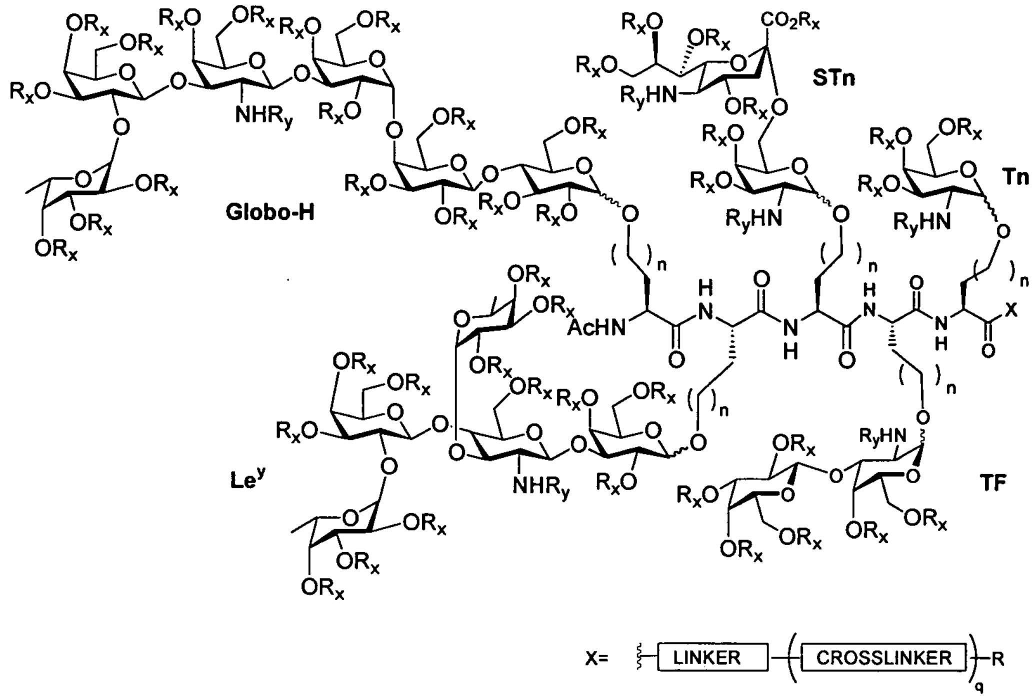
$NH(CH_2)_tNHC(=O)(CH_2)_vS$- wherein t and v are each integers from 1-6. In certain embodiments, one or more of the carbohydrate domains is/are α-linked to the peptide backbone.

[0098] In certain other exemplary embodiments, the present invention provides a novel trimeric antigenic glycopeptide incorporating globo-H, Le^y and Tn, to generate a novel trimeric antigenic compound, as described in more detail below. In certain other exemplary embodiments, the multi-antigenic glycopeptide has the structure:



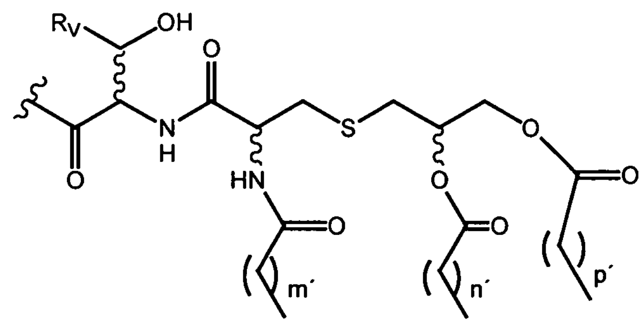
wherein each occurrence of R_x and R_y is independently hydrogen or a protecting group; q is 0 or 1; each occurrence of n is independently an integer from 0-9; R is hydrogen or an immunogenic carrier. In certain exemplary embodiments, the immunogenic carrier is a protein, peptide or lipid. In certain embodiments, each occurrence of n is 3. In certain other embodiments, q is 0. In still other embodiments, the immunogenic carrier is KLH, Human Serum Albumin, Bovine Serum Albumin or polylysine. In certain other embodiments, the immunogenic carrier is PamCys. In yet other embodiments, the linker is a moiety having the structure $-NH(CH_2)_tNHC(=O)(CH_2)_vS$- wherein t and v are each integers from 1-6. In certain embodiments, t is 3 and v is 1. In certain exemplary embodiments, each occurrence of R_x is hydrogen and each occurrence of R_y is Ac. In certain exemplary embodiments, n is 3, q is 0, the immunogenic carrier is KLH or PamCys and the linker is a moiety having the structure $-NH(CH_2)_tNHC(=O)(CH_2)_vS$- wherein t and v are each integers from 1-6. In certain embodiments, one or more of the carbohydrate domains is/are α-linked to the peptide backbone.

[0099] In certain other exemplary embodiments, the present invention provides a novel pentameric antigenic glycopeptide incorporating globo-H, Le^y, STn, TF and Tn to generate a novel pentameric antigenic compound, as described in more detail below. In certain other exemplary embodiments, the multi-antigenic glycopeptide has the structure:



wherein each occurrence of R_x and R_y is independently hydrogen or a protecting group; q is 0 or 1; each occurrence of n is independently an integer from 0-9; R is hydrogen or an immunogenic carrier. In certain exemplary embodiments, the immunogenic carrier is a protein, peptide or lipid. In certain embodiments, each occurrence of n is 3. In certain other embodiments, q is 0. In still other embodiments, the immunogenic carrier is KLH, Human Serum Albumin, Bovine Serum Albumin or polylysine. In certain other embodiments, the immunogenic carrier is PamCys. In yet other embodiments, the linker is a moiety having the structure –$NH(CH_2)_tNHC(=O)(CH_2)_vS$- wherein t and v are each integers from 1-6. In certain embodiments, t is 3 and v is 1. . In certain exemplary embodiments, each occurrence of R_x is hydrogen and each occurrence of R_y is Ac. In certain exemplary embodiments, n is 3, q is 0, the immunogenic carrier is KLH or PamCys and the linker is a moiety having the structure –$NH(CH_2)_tNHC(=O)(CH_2)_vS$- wherein t and v are each integers from 1-6. In certain embodiments, one or more of the carbohydrate domains is/are α-linked to the peptide backbone.

[0100] In certain embodiments, for the multi-antigenic constructs described herein, the immunogenic carrier is a protein, peptide, or lipid. In certain other embodiments of the present invention, R is NHR''', and the carrier R''' is KLH or Bovine Serum Albumin. In still other embodiments of the present invention, R is NHR''', and the carrier R''' is a lipid having the structure:



wherein m′, n′ and p′ are each independently integers between about 8 and 20; and R_V is hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl. In certain exemplary embodiments, m′, n′ and p′ are each 14 and the lipid is tripalmitoyl-S-glycerylcysteinylserine (*e.g.*, PamCys). It will be appreciated that the protein or lipid can be linked to N or the rest of the construct either directly or through a crosslinker and thus R''' incorporates proteins, peptides and lipids, as well as (crosslinker-protein), (crosslinker-peptide) and (crosslinker-lipid) moieties. In certain preferred embodiments, the crosslinker is MMCCH (4-(maleimidomethyl) cyclohexane-1-carboxyl hydrazide).

[0101] Crosslinkers suited to the invention are widely known in the art (see, for example, Appendix A: 1994 Pierce Technical Handbook: cross-linking, also available at www.piercenet.com/resources/browse.cfm?fldID=184), including bromoacetic NHS ester, 6-(iodoacetamido)caproic acid NHS ester, maleimidoacetic acid NHS ester, maleimidobenzoic acide NHS ester, etc. In certain preferred embodiments, the crosslinker is MMCCH (4-(maleimidomethyl) cyclohexane-1-carboxyl hydrazide). In certain other preferred embodiments, the crosslinker is MBS (m-maleimidobenzoyl acid N-Hydroxysuccinimidyl ester).

[0102] For the multi-antigenic constructs described herein, in certain preferred embodiments the carbohydrate determinant is selected from the group consisting of Globo-H, fucosyl GM1, KH-1, N3, glycophorin, Tn, TF, STN, (2,3)ST, 2,6-STn, Gb3, Le^y and Le^x. In

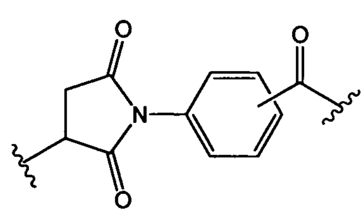
other preferred embodiments, the carbohydrate determinant of the compound contains a Globo-H determinant or fucosyl GM1 determinant as all of part of the carbohydrate determinant A.

[0103] In certain embodiments, for the multi-antigenic constructs described herein, the linker is -O-, $-NR_G-$, $-NR_G$(aliphatic)NR_J-, $-NR_G$(heteroaliphatic)NR_J-, -(aliphatic)NR_J-, -(heteroaliphatic)NR_J-, -O(aliphatic)NR_J-, -O(heteroaliphatic)NR_J-, $-NR_G$(aliphatic)$NR_J(C{=}O)(CR_HR_I)_kS-$, $-NR_G$(heteroaliphatic)$NR_J(C{=}O)(CR_HR_I)_kS-$, -(aliphatic)$NR_J(C{=}O)(CR_HR_I)_kS-$, -(heteroaliphatic)$NR_J(C{=}O)(CR_HR_I)_kS-$, -O(aliphatic)$NR_J(C{=}O)(CR_HR_I)_kS-$, -O(heteroaliphatic)$NR_J(C{=}O)(CR_HR_I)_kS-$, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester, wherein each occurrence of k is independently 1-5; wherein each occurrence of R_G, R_H, R_I or R_J is independently hydrogen, a linear or branched, substituted or unsubstituted, cyclic or acyclic moiety, or a substituted or unsubstituted aryl moiety, and wherein each aliphatic or heteroaliphatic moiety is independently substituted or unsubstituted, linear or branched, cyclic or acyclic.

[0104] In certain embodiments, for the multi-antigenic constructs described herein, the linker is -O-, $-NR_G(CR_HR_I)_kNR_J-$, $-NR_G(CR_HR_I)_kNR_J(C{=}O)(CR_HR_I)_kS-$, $-NR_G-$, $-(CR_HR_J)_kNR_I-$, $-O(CR_HR_I)_kNR_J$, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester, wherein each occurrence of k is independently 1-5, wherein each occurrence of R_G, R_H, R_I or R_J is independently hydrogen, a linear or branched, substituted or unsubstituted, cyclic or acyclic moiety, or a substituted or unsubstituted aryl moiety.

[0105] In certain embodiments, for the multi-antigenic constructs described herein, the linker is a moiety having the structure $-NH(CH_2)_tNHC(=O)(CH_2)_vS-$ wherein t and v are each integers from 1-6. In certain exemplary embodsiments, t is 3 and v is 1.

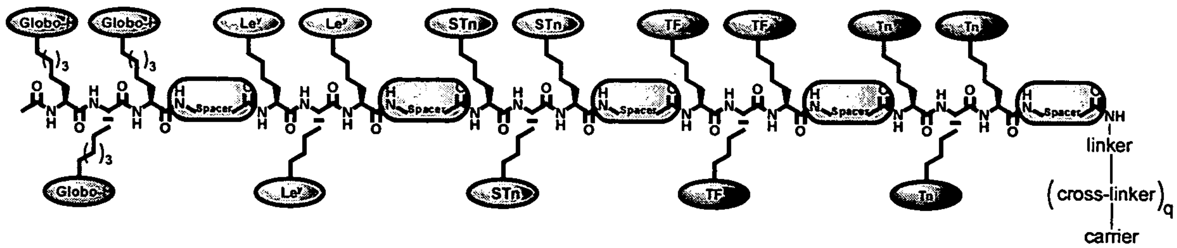
[0106] In certain embodiments for the multi-antigenic constructs described herein, q is 1 and the crosslinker is a fragment having the structure:



;

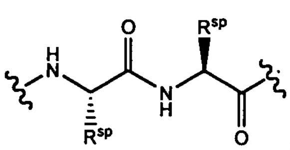
whereby said structure is generated upon conjugation of a maleimidobenzoic acid N-hydroxy succinimide ester with a linker.

[0107] In certain embodiments, the invention provides the clustered multi-antigenic construct having the structure:



wherein q is 0 or 1; the spacer, for each occurrence, is independently a substituted or unsubstituted $C_{1\text{-}6}$alkylidene or $C_{2\text{-}6}$alkenylidene chain wherein up to two non-adjacent methylene units are independently optionally replaced by CO, CO_2, COCO, $CONR^{Z1}$, $OCONR^{Z1}$, $NR^{Z1}NR^{Z2}$, $NR^{Z1}NR^{Z2}CO$, $NR^{Z1}CO$, $NR^{Z1}CO_2$, $NR^{Z1}CONR^{Z2}$, SO, SO_2, $NR^{Z1}SO_2$, SO_2NR^{Z1}, $NR^{Z1}SO_2NR^{Z2}$, O, S, or NR^{Z1}; wherein each occurrence of R^{Z1} and R^{Z2} is independently hydrogen, alkyl, heteroalkyl, aryl, heteroaryl or acyl; a peptidyl moiety or a bivalent aryl or heteroaryl moiety; the linker is either a free carboxylic acid, –O-, (carboxamido)alkyl carboxamide, MBS, primary carboxamide, mono- or dialkyl carboxamide, mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester; and the carrier is an immunogenic carrier.

[0108] In certain exemplary embodiments, each occurrence of the spacer is independently a dipeptidyl moiety. In certain other exemplary embodiments, each occurrence of the spacer is independently a dipeptidyl moiety having the structure:



; wherein R^{sp} is as defined above. In certain embodiments, each occurrence of R^{sp} is independently a natural amino acid side chain. In certain exemplary embodiments, each occurrence of R^{sp} is hydrogen.

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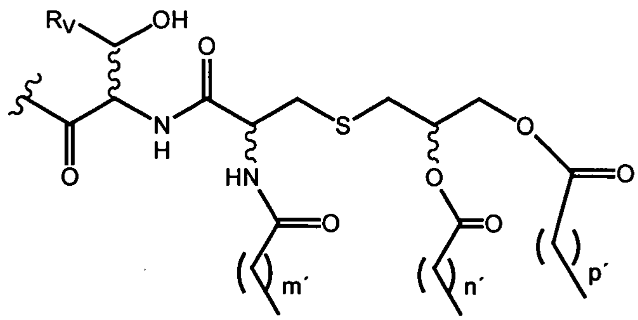
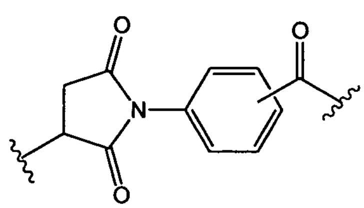
Client Reference No.: SK-893-B-US

[0109] In certain embodiments, the carrier is a protein, peptide or lipid immunogenic carrier. In certain other embodiments of the present invention, the carrier is NHR''', where R''' is KLH or Bovine Serum Albumin. In still other embodiments of the present invention, the carrier is NHR''', where R''' is a lipid having the structure:



wherein m′, n′ and p′ are each independently integers between about 8 and 20; and R_V is hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl. In certain exemplary embodiments, m′, n′ and p′ are each 14 and the lipid is tripalmitoyl-S-glycerylcysteinylserine (*e.g.*, PamCys). It will be appreciated that the protein or lipid can be linked to N or the rest of the construct either directly or through a crosslinker and thus R''' incorporates proteins, peptides and lipids, as well as (crosslinker-protein), (crosslinker-peptide) and (crosslinker-lipid) moieties. In certain preferred embodiments, the crosslinker is MMCCH (4-(maleimidomethyl) cyclohexane-1-carboxyl hydrazide).

[0110] In certain embodiments, q is 1 and the crosslinker is a fragment having the structure:



;

whereby said structure is generated upon conjugation of a maleimidobenzoic acid N-hydroxy succinimide ester with a linker.

[0111] In certain embodiments, the linker is -O-, $-NR_G-$, $-NR_G(\text{aliphatic})NR_J-$, $-NR_G(\text{heteroaliphatic})NR_J-$, $-(\text{aliphatic})NR_J-$, $-(\text{heteroaliphatic})NR_J-$, $-O(\text{aliphatic})NR_J-$, $-O(\text{heteroaliphatic})NR_J-$, $-NR_G(\text{aliphatic})NR_J(C{=}O)(CR_HR_I)_kS-$, $-NR_G(\text{heteroaliphatic})NR_J(C{=}O)(CR_HR_I)_kS-$, $-(\text{aliphatic})NR_J(C{=}O)(CR_HR_I)_kS-$, $-(\text{heteroaliphatic})NR_J(C{=}O)(CR_HR_I)_kS-$, $-O(\text{aliphatic})NR_J(C{=}O)(CR_HR_I)_kS-$, -

O(heteroaliphatic)$NR_J(C{=}O)(CR_HR_I)_kS$-, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester, wherein each occurrence of k is independently 1-5; wherein each occurrence of R_G, R_H, R_I or R_J is independently hydrogen, a linear or branched, substituted or unsubstituted, cyclic or acyclic moiety, or a substituted or unsubstituted aryl moiety, and wherein each aliphatic or heteroaliphatic moiety is independently substituted or unsubstituted, linear or branched, cyclic or acyclic.

[0112] In certain embodiments, the linker is -O-, $-NR_G(CR_HR_I)_kNR_J-$, $-NR_G(CR_HR_I)_kNR_J(C{=}O)(CR_HR_I)_kS-$, $-NR_G-$, $-(CR_HR_J)_kNR_I-$, $-O(CR_HR_I)_kNR_J$, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester, wherein each occurrence of k is independently 1-5, wherein each occurrence of R_G, R_H, R_I or R_J is independently hydrogen, a linear or branched, substituted or unsubstituted, cyclic or acyclic moiety, or a substituted or unsubstituted aryl moiety.

[0113] In certain embodiments, the linker is a moiety having the structure $-NH(CH_2)_tNHC(=O)(CH_2)_vS-$ wherein t and v are each integers from 1-6. In certain exemplary embodsiments, t is 3 and v is 1.

[0114] **2) Synthetic methods**

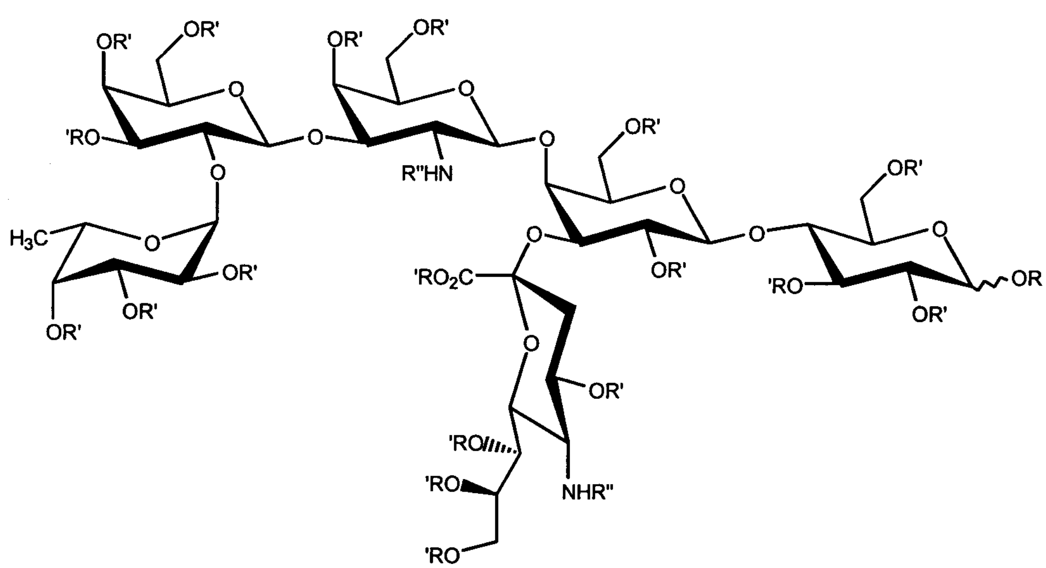
[0115] Tumor-associated carbohydrates

[0116] As mentioned above, the total synthesis of complex antigenic structures has led to significant development in methodologies for complex carbohydrate synthesis. Of particular recent interest is the naturally occurring antigenic structure, fucosylated GM1 ganglioside as shown in Figure 1 (**1a**) which heretofore had not yet been synthesized. Nilsson *et al.* identified fucosyl GM1 as a specific marker associated with small lung cancer (SCLC) cells (Nilsson *et al.*, *Glycoconjugate J.* **1984**, *1*, 43; Brezicka *et al.*, *Cancer Res.* **1989**, *49*, 1300). These workers isolated the glycosphingolipid fucosyl GM1 (**1a**) as the major ganglioside component contained in human SCLC tissue. Furthermore, monoclonal antibodies (F12) to the antigen serve to detect fucosyl GM1 in tissues and serum of SCLC patients (Nilsson *et al.*, *Cancer Res.* **1986**, *46*, 1403; Vangsted *et al.*, *Cancer Res.* **1991**, *51*, 2897). Immunohistochemistry studies have suggested that, due to its highly restricted distribution in normal tissues, fucosyl GM1 could be an excellent

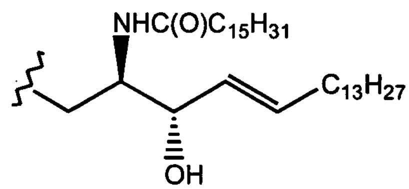
target for immune attack against SCLC. Remarkably, fucosyl GM1 has thus far not been found on any other human cancer cell lines, indicating that it is very SCLC tumor specific (Zhang *et al.*, *Int. J. Cancer* **1997**, *73*, 42).

[0117] The structural assignment of the carbohydrate moiety of the SCLC antigen was based on a combination of enzymatic and chemical degradations (Nilsson *et al.*, *Glycoconjugate J.* **1984**, *1*, 43). While there was no particular reason to question this assignment, the development of a carbohydrate based attack on SCLC could benefit from a definitive assignment of the linkage modes of the various monosaccharides, including the stereochemistry at each glycosidic attachment. Furthermore, no syntheses of this carbohydrate sector have appeared in the literature. In a preferred embodiment, a synthetic scheme would allow for presentation of the hexasaccharide epitope independent of the ceramide to the F12 mAb to ensure that all specificity is directed at the carbohydrate sector. In other preferred embodiments, the construct should be so functionalized as to anticipate the need for its conjugation to a carrier protein in anticipation of building an effective anti-tumor vaccine. As detailed herein, the ability to generate an n-alkenyl glycoside enables for the efficient synthesis of this epitope and allows for its effective modification and/or conjugation to build an effective anti-tumor vaccine.

[0118] Thus, in one aspect of the present invention, the synthesis of the complex fucosyl GM1 carbohydrate sector has been achieved and a compound having the structure as shown below is provided:



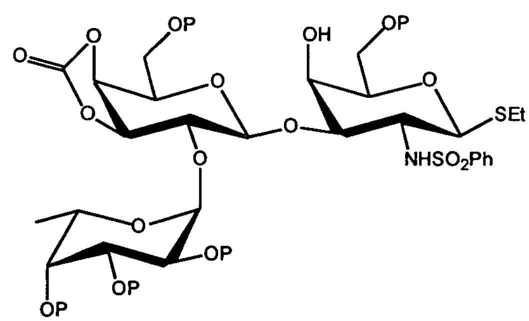
wherein each occurrence of R' is independently hydrogen or a protecting group; wherein each occurrence of R" is independently hydrogen or a nitrogen protecting group; wherein R is hydrogen, substituted or unsubstitued alkyl, alkenyl, -NHR'", wherein R'" is a protein, peptide or lipid linked to N or the rest of the construct either directly or through a crosslinker, an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, which amino acyl moiety or residue, or –NHR'" is linked to O via a polymethylene chain having the structure $-(CH_2)_r$, where r is an integer between 1 and 9, or wherein R is substituted with a moiety having the structure:



[0119] In certain preferred embodiments, each occurrence of R' is hydrogen. In certain other preferred embodiments of the present invention, R is n-alkenyl, including, but not limited to allyl, propenyl, butenyl and pentenyl. In a particularly preferred embodiment, R is n-pentenyl. In certain other preferred embodiments, R is –NHR'", an amino acyl moiety, an amino acyl residue of a peptide, or an amino acyl residue of a protein, as described above, wherein r is preferably 4. In still other preferred embodiments, a compound as described above is provided, with the proviso that the compound is not the glycosphingolipid structure.

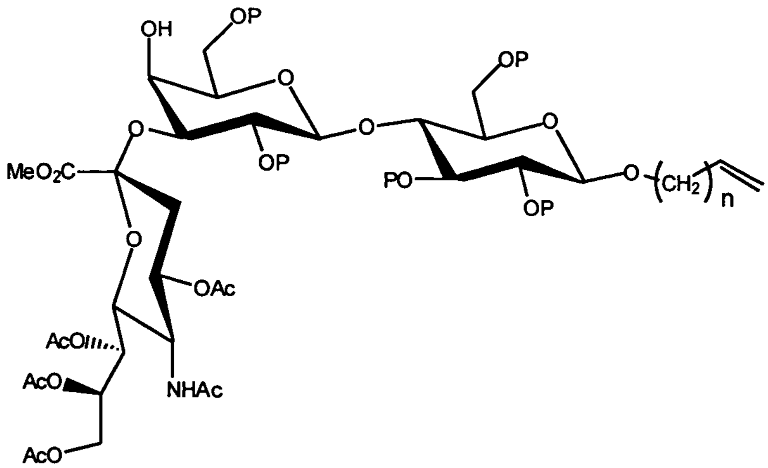
[0120] In another aspect of the present invention, a method for the synthesis of fucosyl GM1 glycoside is provided, said method comprising steps of:

(a) providing a thioethyl donor having the structure:



wherein P is a protecting group;

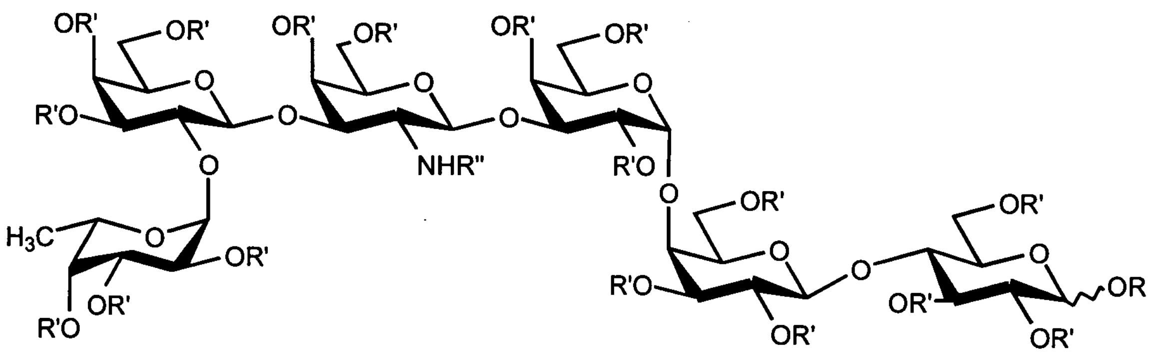
(b) providing a trisaccharide acceptor having the structure:



wherein n is 0-8, and wherein P is a protecting group; and

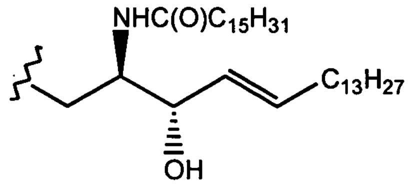
(c) reacting said thioethyl donor and said trisaccharide acceptor under conditions to generate a protected hexasaccharide and subsequently deprotecting the protected hexasaccharide under suitable conditions to generate n-alkenyl fucosyl GM1 glycoside.

[0121] In yet another aspect of the present invention, novel derivatives of Globo-H are provided and a novel general synthetic methodology for the synthesis thereof. The derivatives of Globo-H are depicted below:



wherein each occurrence of R' is hydrogen or a protecting group, wherein R" is hydrogen or a nitrogen protecting group; wherein R is hydrogen, substituted or unsubstitued alkyl or alkenyl, wherein the alkenyl moiety has four or more carbons; -NHR'", wherein R'" is a protein, peptide or lipid linked to N or the rest of the construct either directly or through a crosslinker; an amino acyl moiety; an amino acyl residue of a peptide; an amino acyl residue of a protein; which amino acyl moiety or residue or –NHR'" is linked to O via a polymethylene chain having the structure $-(CH_2)_r$, where, if said carbohydrate moiety is linked to O via an α-linkage, r is an integer between 2 and 9, or, alternatively, if said carbohydrate moiety is linked to O via a β-

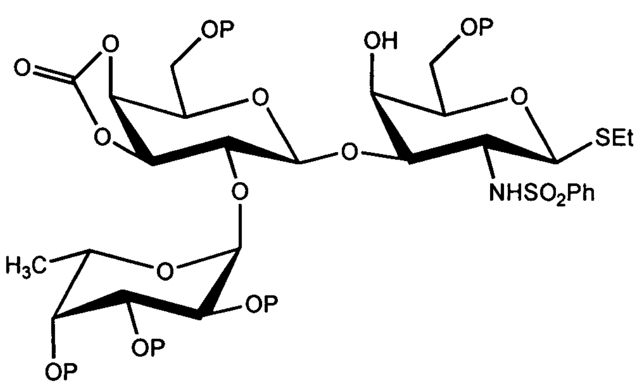
linkage, r is an integer between 1 and 9; or wherein R is substituted with a moiety having the structure:



[0122] In certain preferred embodiments, each occurrence of R' is hydrogen. In certain other preferred embodiments of the present invention, R is n-alkenyl, including, but not limited to allyl, propenyl, butenyl and pentenyl. In a particularly preferred embodiment, R is n-pentenyl. In certain other preferred embodiments, R is an amino acyl moiety, an amino acyl residue of a peptide, or an amino acyl residue of a protein, as described above, wherein r is preferably 4. In still other preferred embodiments, a compound as described above is provided, with the proviso that the compound is not the glycosphingolipid structure.

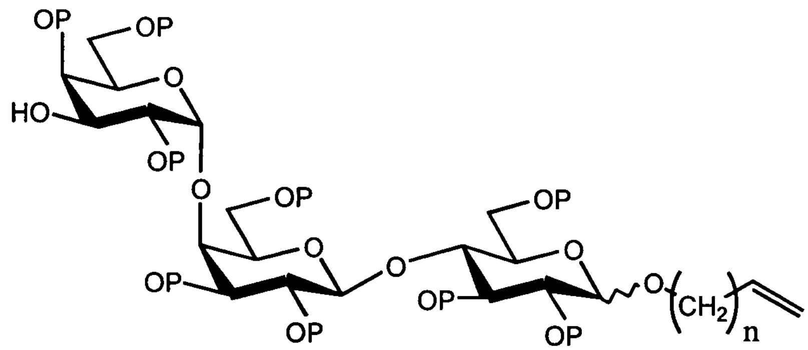
[0123] As described in more detail herein in Example 2, a similar methodology to that described for fucosyl GM1 is employed for the synthesis of Globo-H and derivatives thereof. Thus, in another aspect of the present invention, a method for the improved synthesis of Globo-H, and derivatives thereof, said method comprising steps of:

(a) providing a thioethyl donor having the structure:



wherein P is a protecting group; and

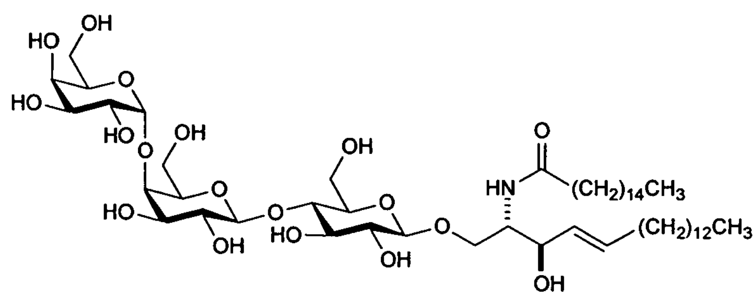
(b) providing a trisaccharide acceptor having the structure:



wherein n is 0-8, and wherein P is a suitable protecting group; and

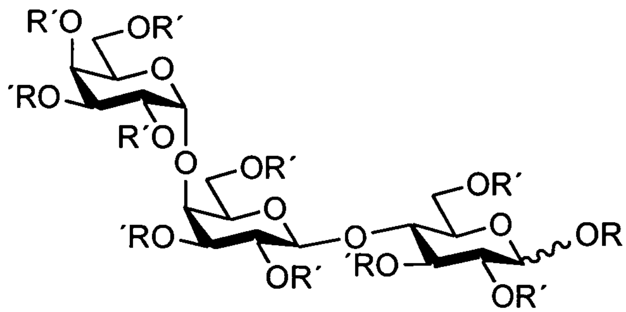
(c) reacting said thioethyl donor and said trisaccharide acceptor under conditions to generate a protected hexasaccharide and subsequently deprotecting the protected hexasaccharide under suitable conditions to generate n-alkenyl Globo-H.

In yet another aspect of the present invention, novel derivatives of Gb3 are provided and a novel general synthetic methodology for the synthesis thereof is described herein. Gb3 is a glycosphingolipid which is structurally related to Globo-H, in that, the trisaccharide portion of Gb3 is identical to the trisaccharide portion at the reducing end of Globo-H. Gb3 has recently been shown to be over expressed in Burkitt lymphoma cell-lines, human ovarian cancer, human teratocarcinoma, human embryonal carcinoma, and other types of tumor cells (See Hashimoto, S.; Sakamoto, H.; Honda, T.; Abe, H.; Nakamura, S.; Ikegami, S. *Tetrahedron Lett.* **1997**, *38*, 8969-8972, and references cited therein). Consequently, Gb3 emerged as an ideal target from the point of view of preparing novel vaccine constructs.

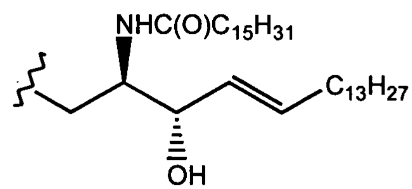


Gb3

[0124] Derivatives of Gb3 provided in the present invention are depicted below:



wherein each occurrence of R' is hydrogen or a protecting group; wherein R is hydrogen, substituted or unsubstitued alkyl or alkenyl; a glycoside-containing moiety; a glycoside-linker moiety; R''', -NHR'''; -(glycoside-linker)-R'''; wherein R''' is an immunogenic carrier linked to the rest of the construct either directly or through a crosslinker; an amino acyl moiety; an amino acyl residue of a peptide; an amino acyl residue of a protein; which amino acyl moiety or residue or –NHR''' is linked to O via a polymethylene chain having the structure $-(CH_2)_r$, where, if said carbohydrate moiety is linked to O via an α-linkage, r is an integer between 2 and 9, or, alternatively, if said carbohydrate moiety is linked to O via a β-linkage, r is an integer between 1 and 9; or wherein R is a moiety having the structure:

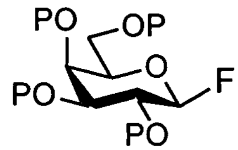


[0125] In certain preferred embodiments, each occurrence of R' is hydrogen. In certain other preferred embodiments of the present invention, R is n-alkenyl, including, but not limited to allyl, propenyl, butenyl and pentenyl. In certain exemplary embodiments, the immunogenic carrier is a protein, peptide or lipid. In a particularly preferred embodiment, R is n-pentenyl. In certain other preferred embodiments, R is an amino acyl moiety, an amino acyl residue of a peptide, or an amino acyl residue of a protein, as described above, wherein r is preferably 4. In certain preferred embodiments, R is a glycoside. In particularly preferred embodiments, R is a monosaccharide. In still other preferred embodiments, a compound as described above is provided, with the proviso that the compound is not the glycosphingolipid structure.

[0126] In another aspect, the invention provides Gb3 n-alkenyl, glycoamino acid, derivatives and conjugates thereof.

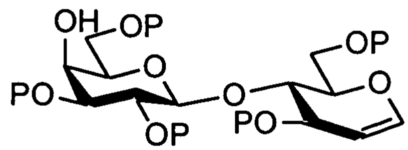
[0127] In another aspect of the present invention, a method for the synthesis of Gb3, and derivatives thereof is provided, said method comprising steps of:

(a) providing a fluoromonosaccharide donor having the structure:



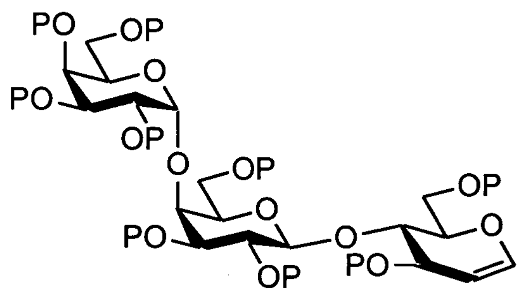
wherein each occurrence of P is independently a suitable protecting group; and

(b) providing a disaccharide acceptor having the structure:



wherein each occurrence of P is independently a suitable protecting group;

(c) reacting said fluoromonosaccharide donor and said disaccharide acceptor under conditions to generate a protected trisaccharide having the structure:

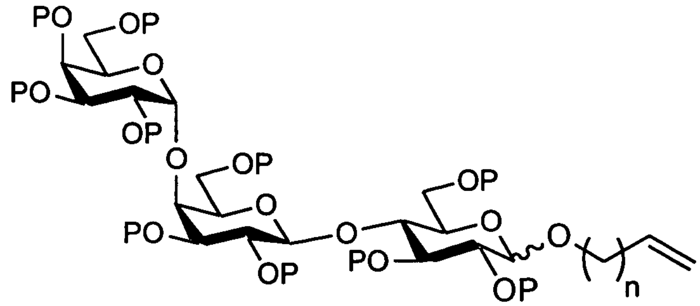


; and

(d) reacting said protected trisaccharide with an alkenol under suitable conditions and subsequently deprotecting the protected hexasaccharide under suitable conditions to generate n-alkenyl Gb3.

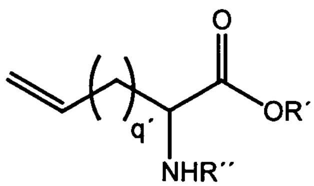
[0128] In another aspect of the present invention, a method for the synthesis of a Gb3 glycoamino acid, and derivatives thereof is provided, said method comprising steps of:

(a) providing an alkenyl trisaccharide having the structure:



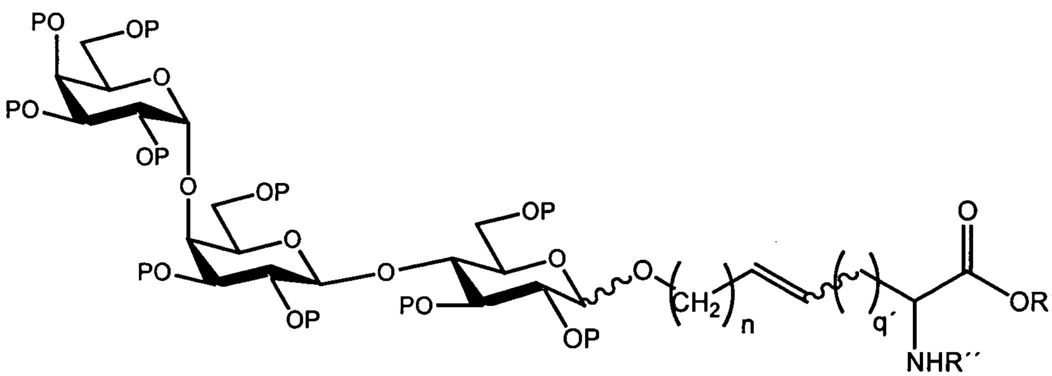
wherein each occurrence of P is independently a suitable protecting group; and n is 1-8;

(b) providing a suitably protected alkenylglycine having the structure:

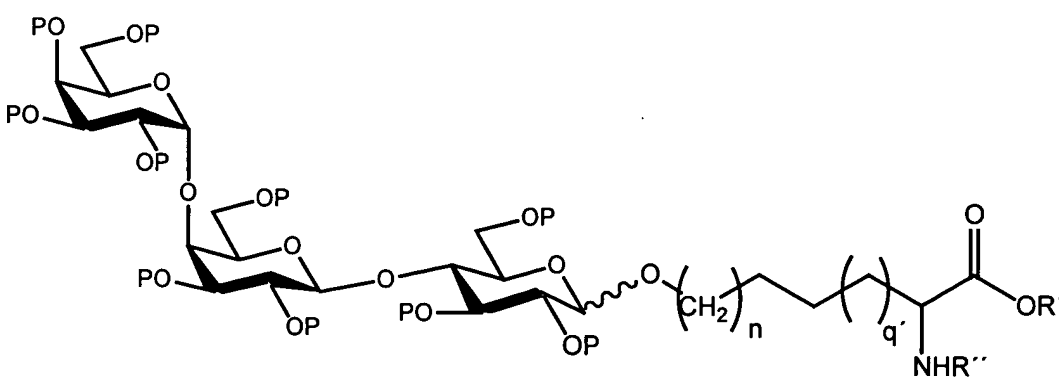


wherein R′ and R′′ are suitable protecting groups; and q′ is 0-8;

(c) reacting said alkenyl trisaccharide and said alkenylglycine under suitable conditions in the presence of a suitable catalyst to generate an enamide ester having the structure:



(c) reacting said enamide ester under suitable conditions to generate a glycoamino acid having the structure:



wherein, for each of the structures above, n is 0-8, q′ is an integer from 0-8, and each occurrence of P, R′ and R′′ is independently a suitable protecting group.

[0129] It will be appreciated that for each of the methods as detailed herein, the full arsenal of protecting groups known in the art of organic synthesis can be utilized, for example, as set forth in "Activating Agents and Protecting Groups: Handbook of Reagents for Organic Synthesis" Roush, W.R. and Pearson, A.J., Eds., John Wiley & Sons: 1999; and "Protective Groups in Organic Synthesis" Greene, T.W. and Wuts, P.G., John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference. In but a few examples, suitable protecting groups utilized herein include, but are not limited to, Bn (benzyl), TIPS (triisopropylsilyl), and Ac (acetate). In a preferred embodiment of the present invention, said thioethyl donor and said trisaccharide acceptor are reacted under MeOTf promotion, as described herein. It will be appreciated by one of ordinary skill in the art however, that a variety of conditions known in the art of organic synthesis can be utilized to effect coupling of these moieties.

[0130] It will also be appreciated that the novel n-alkenyl moieties provided herein can be subsequently modified to generate useful compounds (*e.g.*, alkyl derivatives and glycoamino acids) or constructs thereof (*e.g.*, glycopeptides and conjugated derivatives).

[0131] In addition to providing the first synthesis of fucosyl GM1, a synthesis of Gb3 and improved synthetic methodologies for Globo-H, as described above, in a more general aspect, the present invention provides novel synthetic methodologies for the synthesis of complex carbohydrates comprising (1) providing a carbohydrate acceptor having a reducing end alkenyl group; (2) providing a suitable donor compound and (3) coupling said donor and acceptor under conditions to generate an alkenyl glycoside. Using this method, complex antigenic alkenyl glycosides are provided, as described above, many of which never before have been provided, which can then be conjugated or further reacted, as described herein, to generate glycoconjugates and glycopeptide structures.

[0132] As described above, specifically in the context of the second generation synthesis of the MBr1 antigen (GloboH) and the total synthesis of the fucosylated ganglioside of GM1 (fucosyl GM1) and Gb3, incorporating the reducing end n-alkenyl moiety (specifically n-pentenyl) offers certain benefits. First, the anomeric n-pentenyl glycoside linkage serves as an effective linker for immunoconjugation to carrier protein KLH and also provides some

advantages in terms of synthetic convergency. In the context of protected carbohydrates, the n-alkenyl moieties are also capable of acting as donors for glycosylation (see, for example, Fraser –Reid *et al.*, *SynLett*, **1992**, 927).

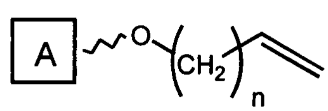
[0133] In this context, the present invention additionally provides methods for the synthesis of n-alkyl glycoamino acids, as described in more detail below for Globo-H and fucosyl GM1 and their subsequent use to generate glycopeptides and synthetic constructs thereof.

[0134] Glycoamino acids

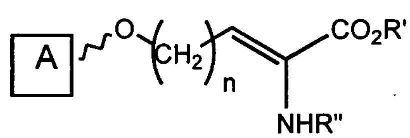
[0135] In certain embodiments, the inventive method for the production of these novel glycoaminoacids comprises: 1) providing an alkenyl glycosidic moiety, as described herein; 2) subjecting said alkenyl glycosidic moiety to oxidative conditions to generate an aldehyde; 3) subjecting said aldehyde to olefination conditions to generate an enamide ester; 4) subjecting said resulting enamide ester to conditions sufficient to hydrogenate said enamide ester to generate a protected glycoamino acid and 5) deprotecting said protected glycoamino acid under suitable conditions to generate a desired glycoamino acid.

[0136] In particular, a method for the synthesis of a glycoamino acid, the structure of which is set forth herein, is provided, which comprises steps of:

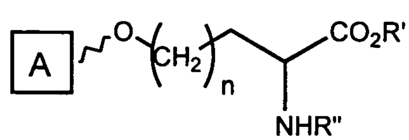
(a) providing an alkenyl glycoside having the structure:



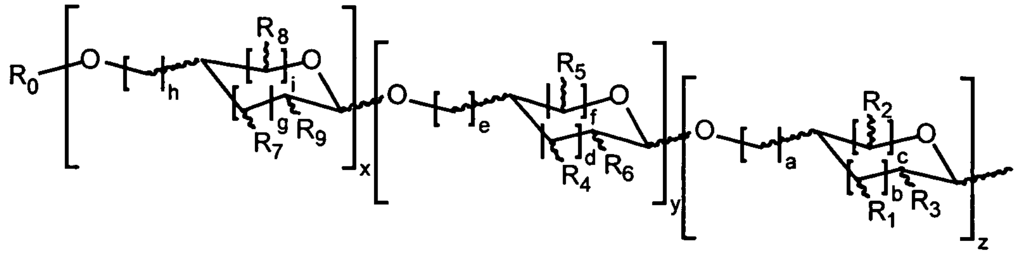
(b) reacting said alkenyl glycoside under suitable conditions to generate an enamide ester having the structure:



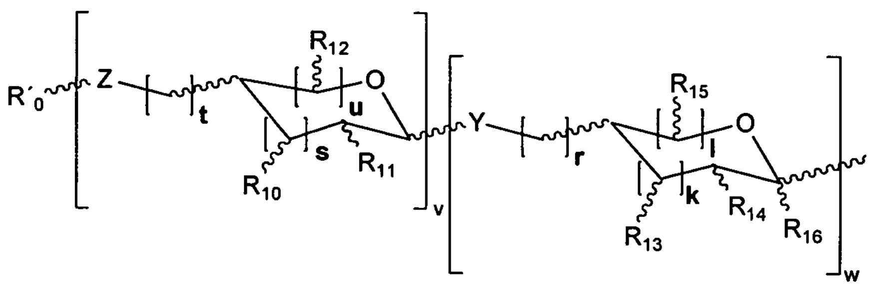
(c) reacting said enamide ester under suitable conditions to generate a glycoamino acid having the structure:



wherein, for each of the structures above, n is 0-8, wherein A is a carbohydrate domain having the structure:



wherein a, b, c, d, e, f, g, h, i, x, y and z are independently 0, 1, 2 or 3, with the proviso that the x, y and z bracketed structures represent furanose or pyranose moieties and the sum of b and c is 1 or 2, the sum of d and f is 1 or 2, and the sum of g and i is 1 or 2, and with the proviso that x, y and z are not simultaneously 0; wherein R_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_1, R_2, R_3, R_4 , R_5, R_6 , R_7, R_8 and R_9 is independently hydrogen, OH, OR^i, NHR^i, $NHCOR^i$, F, CH_2OH, CH_2OR^i, a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i is independently hydrogen, CHO, $COOR^{ii}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group or a saccharide moiety having the structure:



wherein Y and Z are independently NH or O; wherein k, l, r, s, t, u, v and w are each independently 0, 1 or 2; with the proviso that the v and w bracketed structures represent furanose or pyranose moieties and the sum of l and k is 1 or 2, and the sum of s and u is 1 or 2, and with the proviso that v and w are not simultaneously 0; wherein R'_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} is independently hydrogen, OH, OR^{iii}, NHR^{iii}, $NHCOR^{iii}$, F, CH_2OH, CH_2OR^{iii}, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R_{16} is

hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, a substituted or unsubstituted linear or branched chain alkyl or aryl group; wherein each occurrence of R^{iii} is hydrogen, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group; and wherein each occurrence of R^{ii} and R^{iv} are each independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

and wherein for the glycoamino acid structure R' and R" are each independently hydrogen, protecting group, substituted or unsubstituted alkyl, linker, aryl, peptide, protein or lipid; R''' or NHR"', wherein R"' is an immunogenic carrier, protein, peptide, or lipid, linked to N or the rest of the construct either directly or through a crosslinker. In preferred embodiments, R' and R" are each independently hydrogen or a protecting group. In particularly preferred embodiments R" is a nitrogen protecting group, including, but not limited to, acetyl, Fmoc, Cbz or Boc, and R' is an acid protecting group such as benzyl, *t*-butyl or TSE. It will be appreciated, however, that a variety of protecting groups known in the art of organic synthesis can be employed, as referenced herein.

[0137] In certain preferred embodiments, the carbohydrate determinant is selected from the group consisting of Globo-H, fucosyl GM1, KH-1, glycophorin, STN, (2,3)ST, Le^y, Le^x, N3, Tn, 2,6-STn, Gb3 and TF. In certain other preferred embodiments, the carbohydrate determinant of the compound contains a Globo-H determinant or a fucosyl GM1 determinant, as described herein, as all or part of the carbohydrate determinant A.

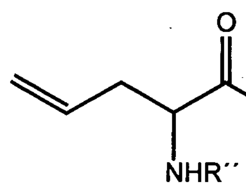
[0138] In general, in preferred embodiments, the step of reacting an n-alkenyl glycoside under suitable conditions to generate an enamide ester comprises reacting an n-alkenyl glycoside first under oxidative conditions and second under olefination conditions in the presence of base (*e.g.*, tetramethylguanidine) and phosphonate to generate an enamide ester. It will be appreciated that other oxidative conditions known in the art of organic synthesis can be employed, including, but not limited to OsO_4 and periodate, or OsO_4 and $Pb(OAc)_4$. Additionally, other well-known bases can be utilized in the present invention, including, but not limited to, lithium t-butoxide or lithium hexamethyl disilylazide.

[0139] In certain exemplary embodiments, reacting said enamide ester under suitable conditions to generate a glycoamino acid comprises reacting said enamide ester under hydrogenation conditions and subsequent reaction under deprotection conditions to generate a glycoamino acid. It is particularly preferred that the hydrogenation conditions employed are

asymmetric hydrogenation conditions. In a preferred embodiment, asymmetric hydrogenation can be achieved by utilizing an ethyl DuPHOS catalyst precursor, as described in more detail herein (see, Burk *et al. Accts. Chem. Res.* **2000,** *33*, 3631; Burk *et al. Pure & Appl. Chem.* **1996,** *68*, 37).

[0140] In certain other embodiments, the inventive method for the production of these novel glycoaminoacids comprises: (i) providing a suitable alkenyl glycoside; (ii) providing a suitably protected alkenylamino acid; (iii) reacting the alkenyl glycoside with the alkenylamino acid under suitable conditions in the presence of a suitable catalyst to generate a glycoenamide ester; and (iv) subjecting the glycoenamide ester to suitable conditions to generate a glycoamino acid.

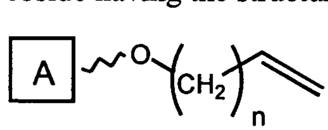
[0141] In preferred embodiments, the step of reacting an alkenyl glycoside under suitable conditions to generate an enamide ester comprises reacting an n-alkenyl glycoside with a suitably protected alkenylamino acid under cross-metathesis conditions in the presence of a suitable catalyst. In certain exemplary embodiments, the protected alkenylamino acid is an allylglycine having the structure:



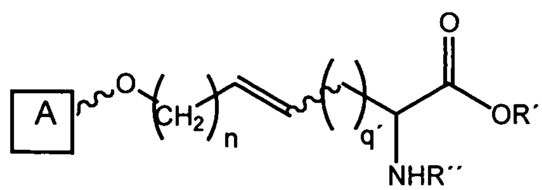
;

wherein R′ and R′′ are each independently hydrogen or a protecting group. In certain embodiments, the step of reacting said glycoenamide ester under suitable conditions to generate a glycoamino acid comprises reacting said enamide ester under hydrogenation conditions and subsequent reaction under deprotection conditions to generate a glycoamino acid. In certain exemplary embodiments, the method for the synthesis of a glycoamino acid comprises steps of:

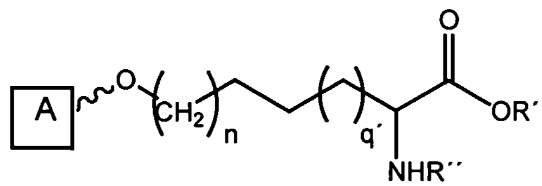
(a) providing an alkenyl glycoside having the structure:



(b) reacting said alkenyl glycosidic moiety with a suitable alkenylglycine under suitable conditions in the presence of a suitable catalyst to generate an enamide ester having the structure:



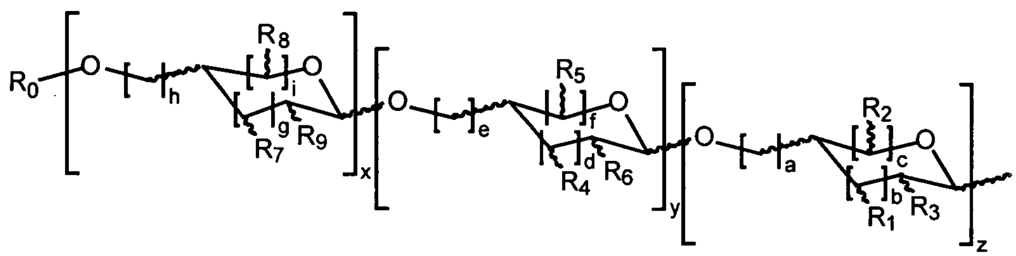
(c) reacting said enamide ester under suitable conditions to generate a glycoamino acid having the structure:



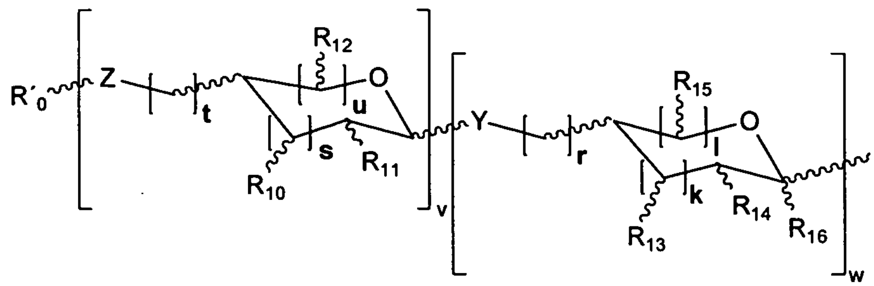
wherein, for each of the structures above, n is 0-8;

q′ is an integer from 0-8;

A is a carbohydrate domain having the structure:



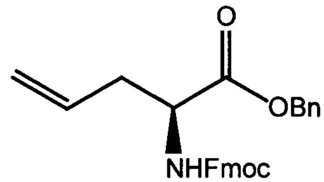
wherein a, b, c, d, e, f, g, h, i, x, y and z are independently 0, 1, 2 or 3, with the proviso that the x, y and z bracketed structures represent furanose or pyranose moieties and the sum of b and c is 1 or 2, the sum of d and f is 1 or 2, and the sum of g and i is 1 or 2, and with the proviso that x, y and z are not simultaneously 0; wherein R_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_1, R_2, R_3, R_4 , R_5, R_6 , R_7, R_8 and R_9 is independently hydrogen, OH, OR^i, NHR^i, $NHCOR^i$, F, CH_2OH, CH_2OR^i, a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i is independently hydrogen, CHO, $COOR^{ii}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group or a saccharide moiety having the structure:



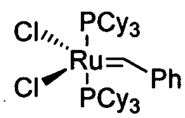
wherein Y and Z are independently NH or O; wherein k, l, r, s, t, u, v and w are each independently 0, 1 or 2; with the proviso that the v and w bracketed structures represent furanose or pyranose moieties and the sum of l and k is 1 or 2, and the sum of s and u is 1 or 2, and with the proviso that v and w are not simultaneously 0; wherein R'_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} is independently hydrogen, OH, OR^{iii}, NHR^{iii}, $NHCOR^{iii}$, F, CH_2OH, CH_2OR^{iii}, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R_{16} is hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, a substituted or unsubstituted linear or branched chain alkyl or aryl group; wherein each occurrence of R^{iii} is hydrogen, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group; and wherein each occurrence of R^{ii} and R^{iv} are each independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

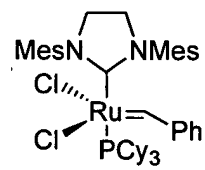
and wherein for the glycoamino acid structure R' and R" are each independently hydrogen, protecting group, substituted or unsubstituted alkyl, linker, aryl, peptide, protein or lipid; R‴ or NHR‴, wherein R‴ is an immunogenic carrier, protein, peptide, or lipid, linked to the rest of the construct directly or through a crosslinker. In preferred embodiments, R′ and R″ are each independently hydrogen or a protecting group. In particularly preferred embodiments R″ is a nitrogen protecting group, including, but not limited to, acetyl, Fmoc, Cbz or Boc, and R′ is an acid protecting group such as benzyl, *t*-butyl or TSE. In certain preferred embodiments, the carbohydrate determinant is selected from the group consisting of Globo-H, fucosyl GM1, KH-1, glycophorin, STN, (2,3)ST, Le^y, Le^x, N3, Tn, 2,6-STn, Gb3 and TF. In certain other preferred embodiments, the carbohydrate determinant of the compound contains a Globo-H determinant or a fucosyl GM1 determinant, as described herein, as all or part of the carbohydrate determinant A.

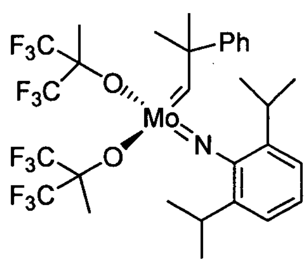
[0142] In certain exemplary embodiments, the protected alkenylglycine has the structure:



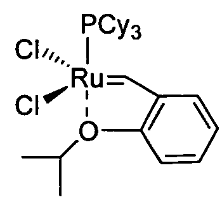
[0143] In certain other embodiments, the catalyst is selected from the group consisting of:







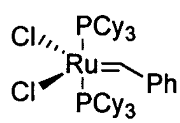
and



;

wherein Cy represents a cyclohexyl moiety. (see, for example, Furstner, A. *Angew. Chem., Int. Ed. Engl.* **2000,** *39*, 3013-3043).

[0144] In certain exemplary embodiments, the catalysts is bis(tricyclohexyl phosphine) ruthenium Grubbs catalyst:



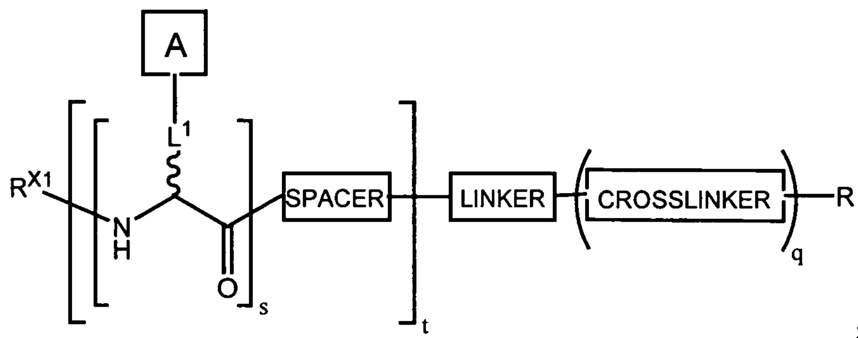
.

[0145] Additionally, in certain embodiments, the step of subjecting the glycoenamide ester to suitable conditions to generate a glycoamino acid comprises catalytic hydrogenation. In certain exemplary embodiments, the hydrogenation reaction concomitantly removes the nitrogen protecting group on the amino acid moiety, thus generating a glycoamino acid unit suitable for incorporation into a peptide construct.

[0146] It will be appreciated that the ability to generate the glycoamino acids, as described herein, ultimately enables the synthesis of novel clustered glycopeptides, a motif commonly found on the surface of cancer cells (mucin-like structures) which are desirable for the uses described herein as anticancer vaccines. For example, immunological studies indicate that, in general, the clustering of antigens in glycopeptides results in a more therapeutically immune response than with singly glycosylated peptides (see, Lo-Man, R. *et al.*, *Cancer Res.*, **1999**, *59*, 1520; Reddish *et al.*, *Glycoconjugate J.* **1997**, *14*, 549).

[0147] Clusters of clusters

[0148] In certain embodiments, the invention provides a method for the production of clustered multi-antigenic constructs having the structure:



;

wherein q is 0 or 1;

each occurrence of s is independently an integer from 2-20;

t is an integer from 1-6;

R^{X1} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a nitrogen protecting group, an amino acid or a proctected amino acid;

R is hydrogen or an immunogenic carrier;

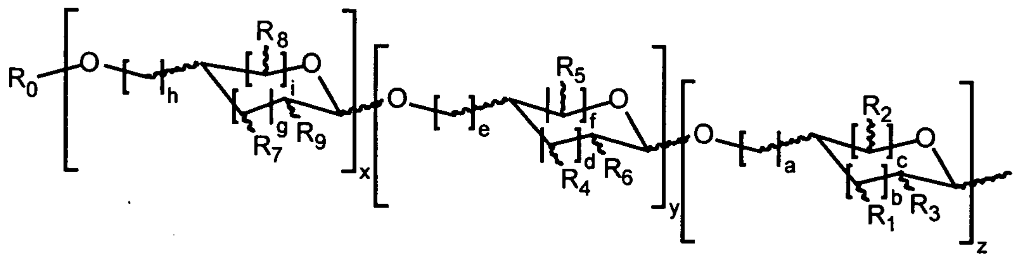
each occurrence of the spacer is independently a substituted or unsubstituted aliphatic, heteroaliphatic, aryl, heteroaryl or peptidic moiety;

the linker is either a free carboxylic acid, –O-, (carboxamido)alkyl carboxamide, MBS, primary carboxamide, mono- or dialkyl carboxamide, mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20

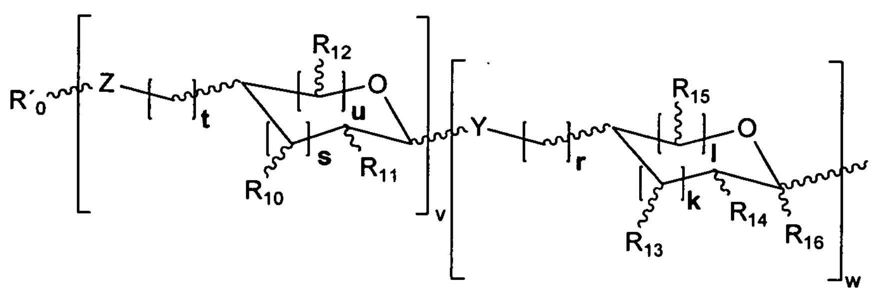
hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester;

each occurrence of L^1 is independently a substituted or unsubstituted aliphatic or heteroaliphatic moiety;

each occurrence of A is independently a carbohydrate domain having the structure:



wherein a, b, c, d, e, f, g, h, i, x, y and z are independently 0, 1, 2 or 3, with the proviso that the x, y and z bracketed structures represent furanose or pyranose moieties and the sum of b and c is 1 or 2, the sum of d and f is 1 or 2, and the sum of g and i is 1 or 2, and with the proviso that x, y and z are not simultaneously 0; wherein R_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_1, R_2, R_3, R_4 , R_5, R_6 , R_7, R_8 and R_9 is independently hydrogen, OH, OR^i, NHR^i, $NHCOR^i$, F, CH_2OH, CH_2OR^i, a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i is independently hydrogen, CHO, $COOR^{ii}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group or a saccharide moiety having the structure:



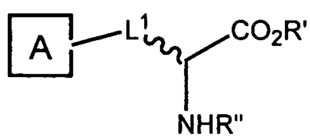
wherein Y and Z are independently NH or O; wherein k, l, r, s, t, u, v and w are each independently 0, 1 or 2; with the proviso that the v and w bracketed structures represent furanose or pyranose moieties and the sum of l and k is 1 or 2, and the sum of s and u is 1 or 2, and with the proviso that v and w are not simultaneously 0; wherein R'_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and

R_{15} is independently hydrogen, OH, OR^{iii}, NHR^{iii}, $NHCOR^{iii}$, F, CH_2OH, CH_2OR^{iii}, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R_{16} is hydrogen, COOH, $COOR^{ii}$, $CONHR^{ii}$, a substituted or unsubstituted linear or branched chain alkyl or aryl group; wherein each occurrence of R^{iii} is hydrogen, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group; and wherein each occurrence of R^{ii} and R^{iv} are each independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; and wherein each glycosidic moiety is either α- or β-linked to an amino acid;

wherein within each bracketed structure s, independently, each occurrence of A is the same.

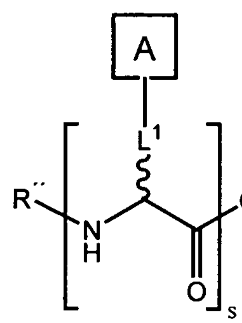
[0149] In certain embodiments, the method comprises steps of:

(a) providing a glycoamino acid having the structure:



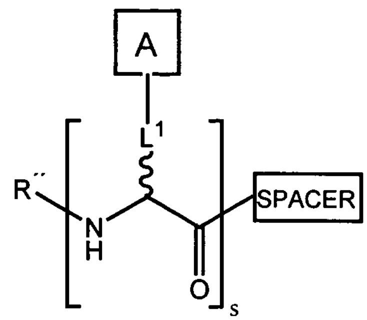
wherein A is a carbohydrate domain as described above;

(b) reacting s occurrences of said glycoamino acid under suitable conditions to generate a glycopeptide having the structure:

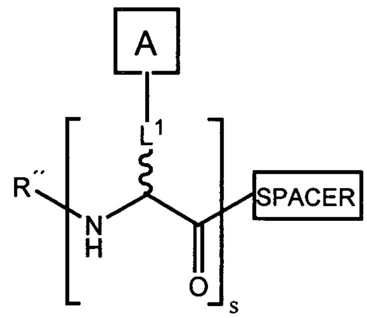


wherein s is an integer from 2-20; each occurrence of A is the same within the bracketed glycopeptide s; R′ is hydrogen or a protecting group; and R′′ is hydrogen, a protecting group, an amino acid or a protected amino acid;

(c) reacting said glycopeptide with a spacer under suitable conditions to generate a spacer construct having the structure:

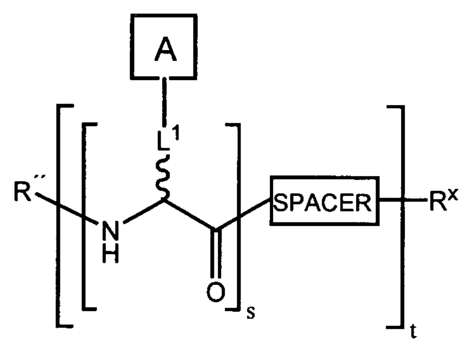


(d) Repeating steps (a) through (c) t-1 times to generate t-1 spacer constructs each independently having the structure:



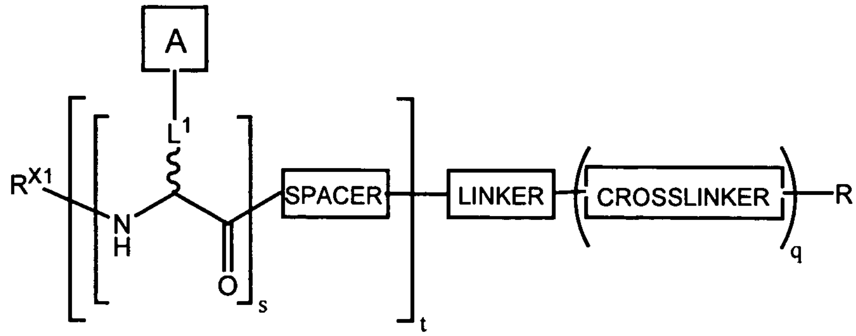
wherein, for each spacer construct, s, L^1, R′′ and the spacer moiety may be the same or different; and each spacer construct comprises a different carbohydrate domain A;

(e) Reacting the spacer construct formed in step (c) with the spacer constructs of step (d) under suitable conditions to generate a construct having the structure:



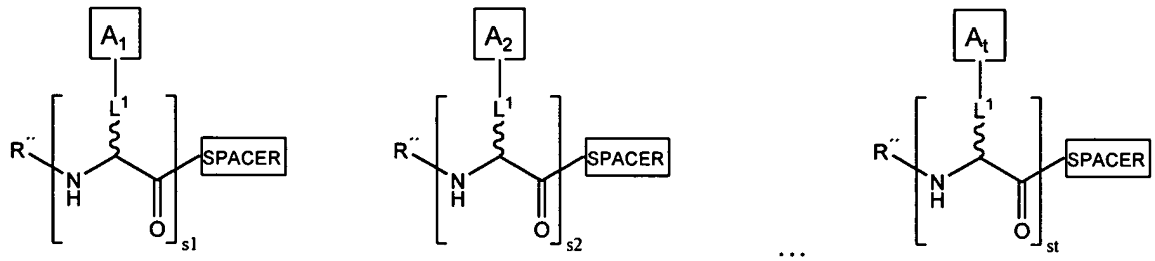
wherein R^x is a protecting group; each occurrence of A is the same within each bracketed structure s; and each bracketed structure s comprises a different carbohydrate domain A; and

(f) Reacting the constructs of step (e) with a linker and optionally a crosslinker and/or an immunogenic carrier under suitable conditions to form the clustered multi-antigenic construct having the structure:



wherein q, linker, crosslinker and R are as defined above.

In certain embodiments, steps (a) through (d) result in the formation of *t* spacer constructs having the structures:



wherein A_1-A_t are different from each other; s1-st are independently integers from 2-20; each occurrence of L^1 may be the same or different; and each occurrence of the spacer may be the same or different.

[0150] The skilled practioner will appreciate that the order with which the different glycopeptide and spacer components are attached together may vary without departing from the scope of the invention. For example, when connecting the glycopeptide clusters together, the approach can be linear or convergent, depending upon the complexity of the target multi-antigenic construct. Additional guidance may be found in Example 6 and in Figures 26-33.

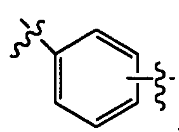
[0151] In certain embodiments, through steps (a)-(f) the carbohydrate domains A may be each, independently, fully protected or deprotected. In certain embodiments, the carbohydrate domain A is in a protected form through steps (a)-(f) and the method further comprises a step of deprotecting each occurrence of A in the multi-antigenic construct. In certain embodiments, the protecting groups present on A are orthogonal to protecting groups R′ and R′′ (*i.e.*, they can be removed/added under reaction conditions that do not affect R′ and/or R′′). In certain exemplary embodiments, the protecting groups present on A include, but are not limited to, acetate, benzoate and methylester. In certain embodiments, R′ comprises -OBz, -OtBu, -OTSE (2-

(trimethylsilyl)ethyl ether) or -OMe. In certain embodiments, R′′ comprises Fmoc, Boc, Cbz or Ac. In certain embodiments, for each occurrence of s, A is selected from the group consisting of Globo-H, fucosyl GM1, KH-1, glycophorin, N3, Tn, TF, STN, (2,3)ST, 2,6-STn, Gb3, Le^y and Le^x.

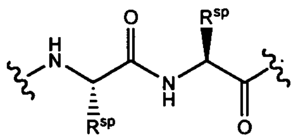
[0152] In certain other embodiments, each occurrence of L^1 is independently a natural amino acid side chain. In certain embodiments, each occurrence of L^1 is independently an unnatural amino acid side chain. In certain embodiments, each occurrence of L^1 is independently $–O(CHR^{aa})_n$- wherein each occurrence of n is independently an integer from 1-10; and each occurrence of R^{aa} is hydrogen, lower alkyl, aryl, heteroaryl, -alkyl(aryl) or –alkyl(heteroaryl). In certain exemplary embodiments, each occurrence of n is 1 and each occurrence of R^{aa} is hydrogen or methyl. In certain embodiments, each occurrence of L^1 is independently a moiety having the structure $–O(CH_2)_n$- wherein n is an integer from 1-10.

[0153] In certain embodiments, R^{X1} is an acyl moiety. In certain exemplary embodiments, R^{X1} is an amino acid residue.

[0154] In certain embodiments, the spacer, for each occurrence, is independently a substituted or unsubstituted C_{1-6}alkylidene or C_{2-6}alkenylidene chain wherein up to two non-adjacent methylene units are independently optionally replaced by CO, CO_2, COCO, $CONR^{Z1}$, $OCONR^{Z1}$, $NR^{Z1}NR^{Z2}$, $NR^{Z1}NR^{Z2}CO$, $NR^{Z1}CO$, $NR^{Z1}CO_2$, $NR^{Z1}CONR^{Z2}$, SO, SO_2, $NR^{Z1}SO_2$, SO_2NR^{Z1}, $NR^{Z1}SO_2NR^{Z2}$, O, S, or NR^{Z1}; wherein each occurrence of R^{Z1} and R^{Z2} is independently hydrogen, alkyl, heteroalkyl, aryl, heteroaryl or acyl; a peptidyl moiety or a bivalent aryl or heteroaryl moiety. In certain exemplary embodiments, the spacer, for each occurrence, is independently $–(CHR^{sp})_n$-, where n is 1-8 and each occurrence of R^{sp} is independently hydrogen, alkyl, cycloalkyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), -OR^{sp1}, -SR^{sp}1 or $–NR^{sp1}R^{sp2}$ where R^{sp1} and R^{sp1} are independently hydrogen or lower alkyl; a peptidyl moiety comprising one or more α-amino acid residues, or a bivalent aryl moiety having the structure:

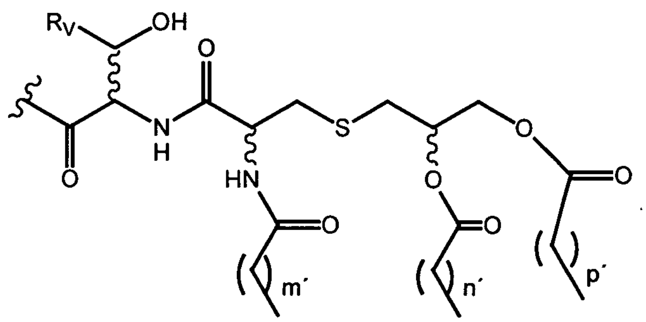


[0155] In certain exemplary embodiments, each occurrence of the spacer is independently a dipeptidyl moiety. In certain other exemplary embodiments, each occurrence of the spacer is independently a dipeptidyl moiety having the structure:



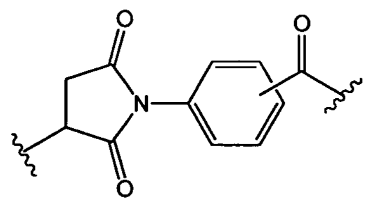
; wherein R^{sp} is as defined above. In certain embodiments, each occurrence of R^{sp} is independently a natural amino acid side chain. In certain exemplary embodiments, each occurrence of R^{sp} is hydrogen.

[0156] In certain embodiments, for the clusters glycopeptides described above and herein, R is a protein, peptide or lipid immunogenic carrier. In certain other embodiments of the present invention, R is NHR''', and the carrier R''' is KLH or Bovine Serum Albumin. In still other embodiments of the present invention, R is NHR''', and the carrier R''' is a lipid having the structure:



[0157] wherein m′, n′ and p′ are each independently integers between about 8 and 20; and R_V is hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl. In certain exemplary embodiments, m′, n′ and p′ are each 14 and the lipid is tripalmitoyl-S-glycerylcysteinylserine (*e.g.*, PamCys). It will be appreciated that the protein or lipid can be linked to N or the rest of the construct either directly or through a crosslinker and thus R''' incorporates proteins, peptides and lipids, as well as (crosslinker-protein), (crosslinker-peptide) and (crosslinker-lipid) moieties. In certain preferred embodiments, the crosslinker is MMCCH (4-(maleimidomethyl) cyclohexane-1-carboxyl hydrazide).

[0158] In certain embodiments for the multi-antigenic constructs described herein, q is 1 and the crosslinker is a fragment having the structure:



;

whereby said structure is generated upon conjugation of a maleimidobenzoic acid N-hydroxy succinimide ester with a linker.

[0159] In certain embodiments, for the multi-antigenic constructs described herein, the linker is -O-, -NR_G-, -NR_G(aliphatic)NR_J-, -NR_G(heteroaliphatic)NR_J-, -(aliphatic)NR_J-, -(heteroaliphatic)NR_J-, -O(aliphatic)NR_J-, -O(heteroaliphatic)NR_J-, -NR_G(aliphatic)NR_J(C=O)$(CR_HR_I)_k$S-, -NR_G(heteroaliphatic)NR_J(C=O)$(CR_HR_I)_k$S-, -(aliphatic)NR_J(C=O)$(CR_HR_I)_k$S-, -(heteroaliphatic)NR_J(C=O)$(CR_HR_I)_k$S-, -O(aliphatic)NR_J(C=O)$(CR_HR_I)_k$S-, -O(heteroaliphatic)NR_J(C=O)$(CR_HR_I)_k$S-, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester, wherein each occurrence of k is independently 1-5; wherein each occurrence of R_G, R_H, R_I or R_J is independently hydrogen, a linear or branched, substituted or unsubstituted, cyclic or acyclic moiety, or a substituted or unsubstituted aryl moiety, and wherein each aliphatic or heteroaliphatic moiety is independently substituted or unsubstituted, linear or branched, cyclic or acyclic.

[0160] In certain embodiments, for the multi-antigenic constructs described herein, the linker is -O-, -$NR_G(CR_HR_I)_kNR_J$-, -$NR_G(CR_HR_I)_kNR_J$(C=O)$(CR_HR_I)_k$S-, -NR_G-, -$(CR_HR_J)_kNR_I$-, -$O(CR_HR_I)_kNR_J$, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester, wherein each occurrence of k is independently 1-5, wherein each occurrence of R_G, R_H, R_I or R_J is independently hydrogen, a linear or branched, substituted or unsubstituted, cyclic or acyclic moiety, or a substituted or unsubstituted aryl moiety.

[0161] In certain embodiments, for the multi-antigenic constructs described herein, the linker is a moiety having the structure –$NH(CH_2)_tNHC(=O)(CH_2)_vS$- wherein t and v are each integers from 1-6. In certain exemplary embodsiments, t is 3 and v is 1.

[0162] 3) Pharmaceutical Compositions

[0163] As described above, the present invention provides compounds and synthetic methodologies useful in the development of novel therapeutic agents, particularly for fully synthetic cancer vaccines and/or therapeutics. In general, the compounds and glycopeptides prepared as disclosed herein can be conjugated to a protein carrier or a lipid to generate useful glycoconjugates for the treatment and/or prevention, (preferably the prevention of the recurrence), of cancer in a subject suffering therefrom. In addition, glycoconjugates prepared by processes disclosed herein are useful in adjuvant therapies as vaccines capable of inducing antibodies immunoreactive with various tumor cells. Such adjuvant therapies may reduce the rate of recurrence of certain cancers, and increase survival rates after surgery. Clinical trials on patients surgically treated for cancer who are then treated with vaccines prepared from a cell surface differentiation antigen found in patients lacking the antibody prior to immunization, a highly significant increase in disease-free interval may be observed. Cf. P.O. Livingston, *et al., J. Clin. Oncol.*, **1994**, *12*, 1036.

[0164] Thus, the present invention provides pharmaceutical compositions for treating cancer, preferably for preventing the recurrence of cancer, comprising any of the compounds of the present invention disclosed herein, as an active ingredient, optionally, though typically in combination with a pharmaceutically acceptable carrier. The pharmaceutical compositions of the present invention may further comprise other therapeutically active ingredients (*e.g.*, chemotherapeutic and/or palliative). For purposes of the invention, the term *"Palliative"* refers to treatment that is focused on the relief of symptoms of a disease and/or side effects of a therapeutic regimen, but is not curative. For example, palliative treatment encompasses painkillers, antinausea medications and anti-sickness drugs. In addition, chemotherapy, radiotherapy and surgery can all be used palliatively (that is, to reduce symptoms without going for cure; *e.g.*, for shrinking tumors and reducing pressure, bleeding, pain and other symptoms of cancer).

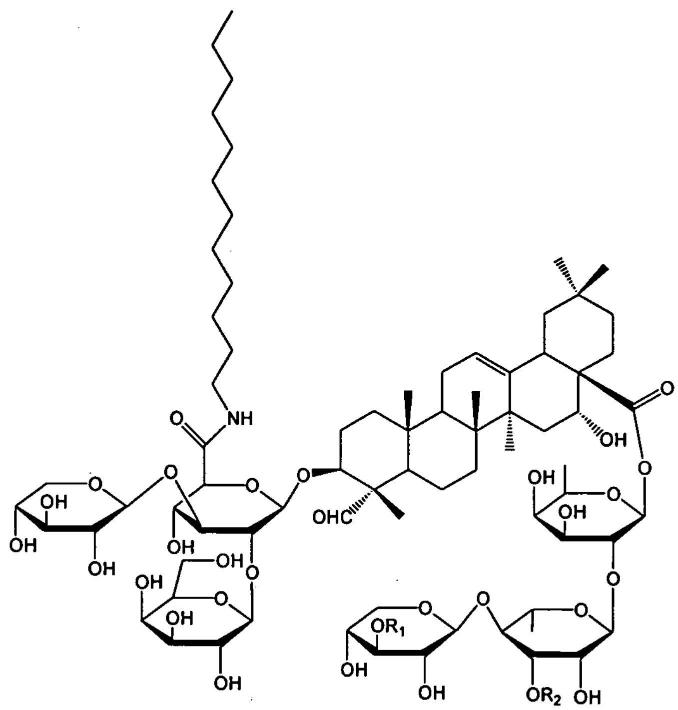
[0165] In preparing oral dosage forms, any of the unusual pharmaceutical media may be used, such as water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like in the case of oral liquid preparations (*e.g.*, suspensions, elixers and solutions); or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disinterating agents, etc., in the case of oral solid preparations are preferred

over liquid oral preparations such as powders, capsules and tablets. If desired, capsules may be coated by standard aqueous or non-aqueous techniques. In addition to the dosage forms described above, the compounds of the invention may be administered by controlled release means and devices.

[0166] As described above, the pharmaceutical compositions of the present invention additionally comprise a pharmaceutically acceptable carrier, which, as used herein, includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's Pharmaceutical Sciences, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980) discloses various carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is incompatible with the compounds of the invention, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention. Some examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatine; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil, sesame oil; olive oil; corn oil and soybean oil; glycols; such as propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogenfree water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator.

[0167] In certain embodiments, pharmaceutical compositions of the invention comprise an immunological adjuvant, or a combination of immunological adjuvants.

[0168] In certain embodiments, the adjuvant is a saponin adjuvant (see, *e.g.*, Marciani *et al.*, *Vaccine*, **2000**, *18*, 3141, US Patent No.: 6,080,725 and 5,977,081, the entire contents of which are hereby incorporated by reference). One example of a preferred saponin adjuvant includes, but is not limited to, GPI-0100, (Galenica Pharmaceuticals, Inc., Frederick, MD) which is a semi-synthetic adjuvant derived by modifying selected natural saponins.



GPI-0100

[0169] Saponins isolated from *Quillaja soponaria Molina* contain two acyl moieties, a normonoterpene carboxylic acid and a normonoterpene carboxylic acid glycoside, which are linked linearly to a fucosyl residue attached at position C-28. It has been hypothesized that these lipophilic acyl groups may be responsible for these saponins' toxicity and their ability to stimulate cytotoxic T cells against exogenous antigens. The linkage between the fucosyl residue and the acyl group is unstable and hydrolyzes under mild conditions (pH≥6) with concomittant loss of saponins capability to stimulate cell-mediated immune response. Unlike their saponin precursors, GPI-0100 adjuvants comprise a stable non-toxic lipophilic moiety in the saponin's glucuronic residue. Methods for preparing these semi-synthetic adjuvants are well-known in the art. For example, GPI-0100 adjuvants may be prepared by hydrolizing quillaja saponins (which are commercially available) under basic conditions to yield the corresponding deacylated

product. The deacylated intermediate may then be reacted with a suitable amine reagent using standard carboxylic acid moiety activation methodology to give the desired compounds. A wide variety of procedures are effective for extrating saponin compounds. They are generalized as follows: (i) defatting of the organic matter with a hydrophobic organic solvent such as petroleum ether; (ii) extraction with a suitable alcohol (*e.g.*, methanol or ethanol) or alcohol-water mixture; (iii) evaporation of the carinol solvent; and (iv) partitioning of the dried alcohol extract between water and n-butanol saturated with water, followed by precipitation of the crude saponins from the n-butanol/water with a suitable organic solvent (*e.g.*, diethyl ether). Purification of the saponin extract may require multiple separation steps. For example, preliminary fractionation may be carried out using conventional open column chromatography or flash chromatography on silica gel, in combination with a more sophisticated chromatographic technique such as High Pressure Liquid Chromatography (HPLC), droplet counter-current liquid chromatography (DCCC) or centrifugal Liquid Chromatography (RLCC). The integration of these techniques with preparative TLC typically affords separated and purified saponins.

[0170] In certain other embodiments, the adjuvant is bacteria or liposomes. In certain exemplary embodiments, the adjuvant includes but is not limited to, *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21.

[0171] Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

[0172] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for

example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

[0173] The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[0174] In order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension or crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution that, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include (poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions, which are compatible with body tissues.

[0175] Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

[0176] Solid dosage forms (e.g., for oral administration) include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin,

polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar--agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

[0177] Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polethylene glycols and the like.

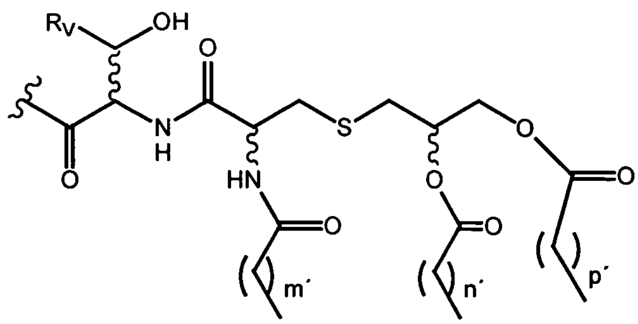
[0178] The active compounds can also be in microencapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose and starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, *e.g.*, tableting lubricants and other tableting aids such as magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the activê ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner.

Examples of embedding compositions, which can be used, include polymeric substances and waxes.

[0179] Pharmaceutical compositions of the present invention suitable for oral administration may be prepared as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient in powder or granular form or as a solution or suspension in an aqueous or nonaqueous liquid or in an oil-in-water or water-in-oil emulsion. Such compositions may be prepared by any of the methods known in the art of pharmacy. In general, compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers, finely divided solid carriers, or both and then, if necessary, shaping the product into the desired form. For example, a tablet may be prepared by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granule optionally mixed with a binder, lubricant, inert diluent or surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent.

[0180] 4) Pharmaceutical Uses and Methods of Treatment

[0181] In certain embodiments, a method of treatment is provided comprising administering to the subject a therapeutically effective amount of any of the glyconjugates disclosed herein, optionally in combination with a pharmaceutically acceptable carrier. The method may be applied wherein the cancer is a solid tumor or an epithelial tumor. As mentioned above, methods for the treatment of cancer (preferably for the prevention of recurrence of cancer) are provided, as well as methods for inducing antibodies in a human subject, wherein the antibodies are capable of specifically binding with human tumor cells, which comprises administering to the subject an amount of any of the glycoconjugates disclosed above effective to induce antibodies. In certain embodiments, the carbohydrate antigen is linked to an immunogenic carrier either directly or through a crosslinker, which carrier is a protein, peptide or lipid. In certain embodiments, the carrier is Human Serum Albumin, Bovine Serum Albumin, polylysine or KLH. In certain other embodiments, the carrier is a lipid having the structure:

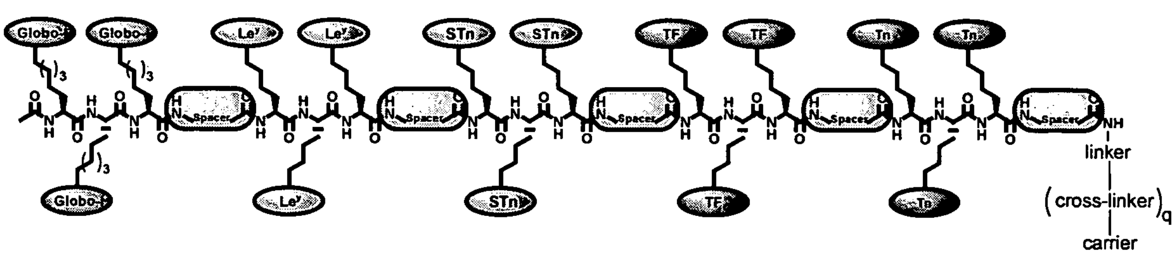


wherein m′, n′ and p′ are each independently integers between about 8 and 20; and R_V is hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl. In certain exemplary embodiments, m′, n′ and p′ are each 14 and the lipid is tripalmitoyl-S-glycerylcysteinylserine (*e.g.*, PamCys).

[0182] In certain other embodiments, the method comprises administering to the subject a therapeutically effective amount of any of the compounds and/or glycopeptides disclosed herein, in combination with an immunogenic carrier, optionally in combination with a pharmaceutically acceptable carrier. Specifically, in certain exemplary embodiments, the method comprises administering a carbohydrate antigen conjugated to an immunogenic carrier. In certain embodiments, the method comprises administering a carbohydrate antigen and an immunogenic carrier that have not been conjugated. Rather, they are administered concurrently, or successively, as separate entities. In certain other exemplary embodiments, the method comprises administering a glycopeptide of the invention conjugated to an immunogenic carrier. In certain embodiments, the method comprises administering an inventive glycopeptide that has not been conjugated to an immunogenic carrier. Rather, the glycopeptide and the immunogenic carrier are administered concurrently, or successively, as separate entities. In certain embodiments, the immunogenic carrier is a protein, pepitde or lipid. In certain exemplary embodiments, the carrier is Human Serum Albumin, Bovine Serum Albumin, polylysine or KLH. In certain other embodiments, the carrier is PamCys. For the purpose of the invention, a compound/glycopeptide and a carrier are said to be administrered concurrently when they are administered (i) as a single composition containing the compound/glycopeptide and the carrier, (ii) as two separate compositions or (iii) are delivered by separate routes within a short enough period of time that the effective result is equivalent to that obatined when both compound/glycopeptide and carrier are administered as a single composition.

[0183] In still other embodiments, the present invention provides the related method of inducing antibodies which further comprises co-administering an immunological adjuvant, or a combination of immunological adjuvants. In certain embodiments, the adjuvant is a saponin adjuvant. In certain other embodiments, the adjuvant is bacteria or liposomes. In certain exemplary embodiments, the adjuvant includes but is not limited to, *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21. Specifically, when a multi-antigenic glycopeptide comprising at least two different antigenic carbohydrate domains is used, it is possible to induce at least two different types of antibodies. In certain embodiments, each carbohydrate antigen present on the peptide backbone elicits an antibody type specific to that carbohydrate antigen. In certain other embodiments, the antibodies produced are those that recognize at least one carbohydrate antigen present on the glycopeptide. In certain embodiments, an inventive multi-antigenic glycopeptide, when administered to a subject, produces antibodies to a subset of the carbohydate antigens present on the glycopeptide backbone. In certain embodiments, some of the antibodies produced recognize two or more carbohydrate antigens of the glycopeptide. In certain exemplary embodiements, the inventive glycopeptides comprise carbohydrate domains, or truncated or elongated versions thereof, that are found on tumor cells.

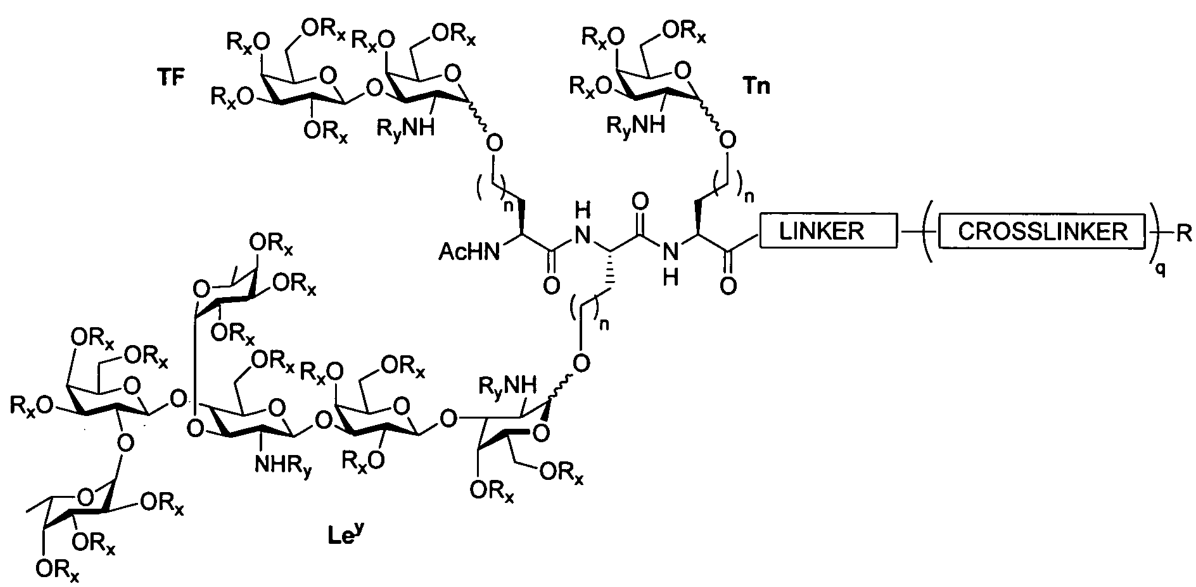
[0184] In certain exemplary embodiments, the multi-antigenic glycopeptide is a clustered construct having the structure:



wherein q is 0 or 1; the spacer, for each occurrence, is independently a substituted or unsubstituted C_{1-6}alkylidene or C_{2-6}alkenylidene chain wherein up to two non-adjacent methylene units are independently optionally replaced by CO, CO_2, COCO, $CONR^{Z1}$, $OCONR^{Z1}$, $NR^{Z1}NR^{Z2}$, $NR^{Z1}NR^{Z2}CO$, $NR^{Z1}CO$, $NR^{Z1}CO_2$, $NR^{Z1}CONR^{Z2}$, SO, SO_2, $NR^{Z1}SO_2$, SO_2NR^{Z1}, $NR^{Z1}SO_2NR^{Z2}$, O, S, or NR^{Z1}; wherein each occurrence of R^{Z1} and R^{Z2} is independently hydrogen, alkyl, heteroalkyl, aryl, heteroaryl or acyl; a peptidyl moiety or a bivalent aryl or heteroaryl moiety; the linker is either a free carboxylic acid, –O-, (carboxamido)alkyl carboxamide, MBS, primary carboxamide, mono- or dialkyl carboxamide,

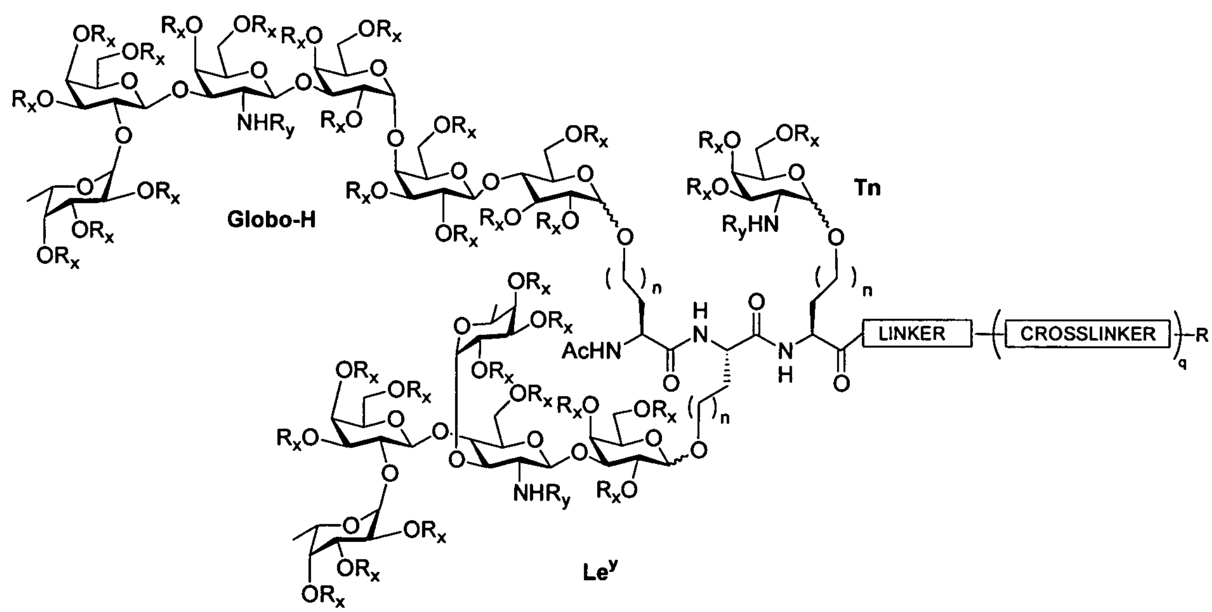
mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester; and the carrier is an immunogenic carrier. In certain exemplary embodiments, the immunogenic carrier is a protein, peptide or lipid. In certain other embodiments, q is 0. In still other embodiments, the immunogenic carrier is KLH, Human Serum Albumin, Bovine Serum Albumin or polylysine. In certain other embodiments, the immunogenic carrier is PamCys. In yet other embodiments, the linker is a moiety having the structure –$NH(CH_2)_tNHC(=O)(CH_2)_vS$- wherein t and v are each integers from 1-6. In certain embodiments, t is 3 and v is 1. In certain exemplary embodiments q is 0, the immunogenic carrier is KLH or PamCys and the linker is a moiety having the structure –$NH(CH_2)_tNHC(=O)(CH_2)_vS$- wherein t and v are each integers from 1-6. In certain embodiments, one or more of the carbohydrate domains is/are α-linked to the peptide backbone. In certain embodiments, one or more of the carbohydrate domains is/are α-O-linked to the peptide backbone.

[0185] In certain exemplary embodiments, the multi-antigenic glycopeptide is a trimeric construct having the structure:



wherein each occurrence of R_x and R_y is independently hydrogen or a protecting group; q is 0 or 1; each occurrence of n is independently an integer from 0-9; R is hydrogen or an immunogenic carrier. In certain exemplary embodiments, the immunogenic carrier is a protein, peptide or lipid. In certain embodiments, each occurrence of n is 0. In certain other embodiments, q is 0. In still other embodiments, the immunogenic carrier is KLH, Human Serum Albumin, Bovine Serum Albumin or polylysine. In certain other embodiments, the immunogenic carrier is PamCys. In yet other embodiments, the linker is a moiety having the structure –$NH(CH_2)_tNHC(=O)(CH_2)_vS$- wherein t and v are each integers from 1-6. In certain embodiments, t is 3 and v is 1. In certain exemplary embodiments, each occurrence of R_x is hydrogen and each occurrence of R_y is Ac. In certain exemplary embodiments, n and q are each 0, the immunogenic carrier is KLH or PamCys and the linker is a moiety having the structure –$NH(CH_2)_tNHC(=O)(CH_2)_vS$- wherein t and v are each integers from 1-6. In certain embodiments, one or more of the carbohydrate domains is/are α-linked to the peptide backbone.

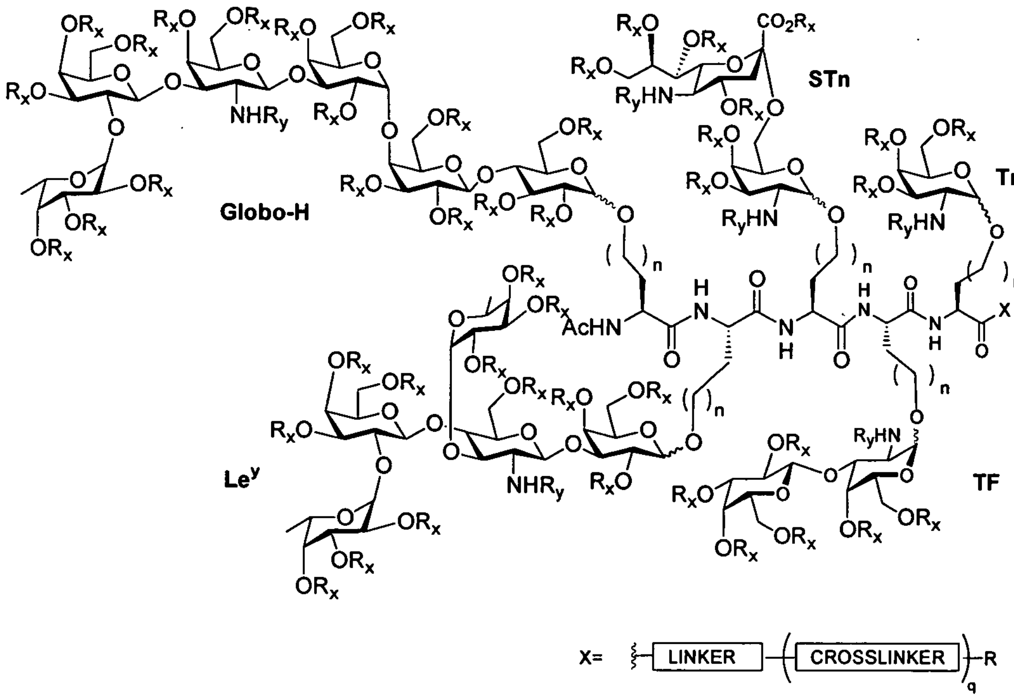
[0186] In certain other exemplary embodiments, the multi-antigenic glycopeptide has the structure:



wherein each occurrence of R_x and R_y is independently hydrogen or a protecting group; q is 0 or 1; each occurrence of n is independently an integer from 0-9; R is hydrogen or an immunogenic carrier. In certain exemplary embodiments, the immunogenic carrier is a protein, peptide or lipid. In certain embodiments, each occurrence of n is 3. In certain other

embodiments, q is 0. In still other embodiments, the immunogenic carrier is KLH, Human Serum Albumin, Bovine Serum Albumin or polylysine. In certain other embodiments, the immunogenic carrier is PamCys. In yet other embodiments, the linker is a moiety having the structure $-NH(CH_2)_tNHC(=O)(CH_2)_vS-$ wherein t and v are each integers from 1-6. In certain embodiments, t is 3 and v is 1 In certain exemplary embodiments, each occurrence of R_x is hydrogen and each occurrence of R_y is Ac. In certain exemplary embodiments, n is 3, q is 0, the immunogenic carrier is KLH or PamCys and the linker is a moiety having the structure $-NH(CH_2)_tNHC(=O)(CH_2)_vS-$ wherein t and v are each integers from 1-6. In certain embodiments, one or more of the carbohydrate domains is/are α-linked to the peptide backbone.

[0187] In certain other exemplary embodiments, the multi-antigenic glycopeptide has the structure:



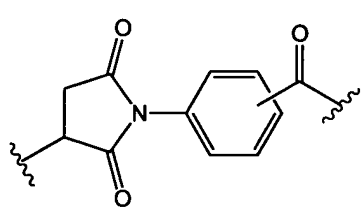
wherein each occurrence of R_x and R_y is independently hydrogen or a protecting group; q is 0 or 1; each occurrence of n is independently an integer from 0-9; R is hydrogen or an immunogenic carrier. In certain exemplary embodiments, the immunogenic carrier is a protein, peptide or lipid. In certain embodiments, each occurrence of n is 3. In certain other embodiments, q is 0. In still other embodiments, the immunogenic carrier is KLH, Human Serum Albumin, Bovine Serum Albumin or polylysine. In certain other embodiments, the immunogenic carrier is PamCys. In yet other embodiments, the linker is a moiety having the structure –

$NH(CH_2)_tNHC(=O)(CH_2)_vS$- wherein t and v are each integers from 1-6. In certain embodiments, t is 3 and v is 1. In certain exemplary embodiments, each occurrence of R_x is hydrogen and each occurrence of R_y is Ac. In certain exemplary embodiments, n is 3, q is 0, the immunogenic carrier is KLH or PamCys and the linker is a moiety having the structure –$NH(CH_2)_tNHC(=O)(CH_2)_vS$- wherein t and v are each integers from 1-6. In certain embodiments, one or more of the carbohydrate domains is/are α-linked to the peptide backbone.

[0188] In certain embodiments, for each of the above three constructs, the linker is -O-, -NR_G-, -NR_G(aliphatic)NR_J-, -NR_G(heteroaliphatic)NR_J-, -(aliphatic)NR_J-, -(heteroaliphatic)NR_J-, -O(aliphatic)NR_J-, -O(heteroaliphatic)NR_J-, -NR_G(aliphatic)$NR_J(C=O)(CR_HR_I)_kS$-, -NR_G(heteroaliphatic)$NR_J(C=O)(CR_HR_I)_kS$-, -(aliphatic)$NR_J(C=O)(CR_HR_I)_kS$-, -(heteroaliphatic)$NR_J(C=O)(CR_HR_I)_kS$-, -O(aliphatic)$NR_J(C=O)(CR_HR_I)_kS$-, -O(heteroaliphatic)$NR_J(C=O)(CR_HR_I)_kS$-, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester, wherein each occurrence of k is independently 1-5; wherein each occurrence of R_G, R_H, R_I or R_J is independently hydrogen, a linear or branched, substituted or unsubstituted, cyclic or acyclic moiety, or a substituted or unsubstituted aryl moiety, and wherein each aliphatic or heteroaliphatic moiety is independently substituted or unsubstituted, linear or branched, cyclic or acyclic.

[0189] In certain embodiments, for each of the above three constructs, the linker is -O-, -$NR_G(CR_HR_I)_kNR_J$-, -$NR_G(CR_HR_I)_kNR_J(C=O)(CR_HR_I)_kS$-, -$NR_G$-, -$(CR_HR_J)_kNR_I$-, -$O(CR_HR_I)_kNR_J$, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester, wherein each occurrence of k is independently 1-5, wherein each occurrence of R_G, R_H, R_I or R_J is independently hydrogen, a linear or branched, substituted or unsubstituted, cyclic or acyclic moiety, or a substituted or unsubstituted aryl moiety.

[0190] In certain embodiments, for each of the above three constructs, q is 1 and the crosslinker is a fragment having the structure:


;

whereby said structure is generated upon conjugation of a maleimidobenzoic acid N-hydroxy succinimide ester with a linker.

[0191] It will be appreciated that the magnitude of the therapeutic dose of the compounds of the invention will vary with the nature and severity of the condition to be treated and with the particular compound and its route of administration. In general, the daily dose range for anticancer activity lies in the range of 0.0001 to 1.0 mg/kg of body weight in a mammal, although the present invention is not intended to be limited by this range.

[0192] Any suitable route of administration may be employed for providing a mammal, especially a human, with an effective dosage of a compound disclosed herein. For example, oral, rectal, topical, parenteral, ocular, pulmonary, nasal, etc. routes may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, etc. In preferred embodiments, the effective dosage is employed using a syringe injection.

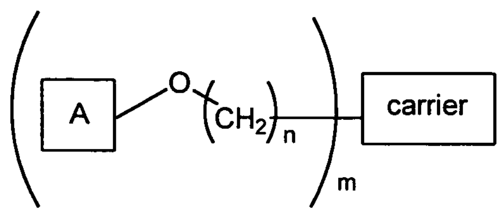
[0193] The inventive compositions include those suitable for oral, rectal, topical (including transdermal devices, aerosols, creams, ointments, lotions and dusting powders), parenteral (including subcutaneous, intramuscular, and intravenous), ocular (opthalmic), pulmonary (nasal or buccal inhalation) or nasal administration. Although the most suitable route in any given case will depend largely on the nature and severity of the condition being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

[0194] It will be appreciated by one of ordinary skill in the art, however, that the most suitable route for administration will depend largely on the nature and severity of the condition being treated and on the nature of the active ingredient. As discussed above, the inventive therapeutics may be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

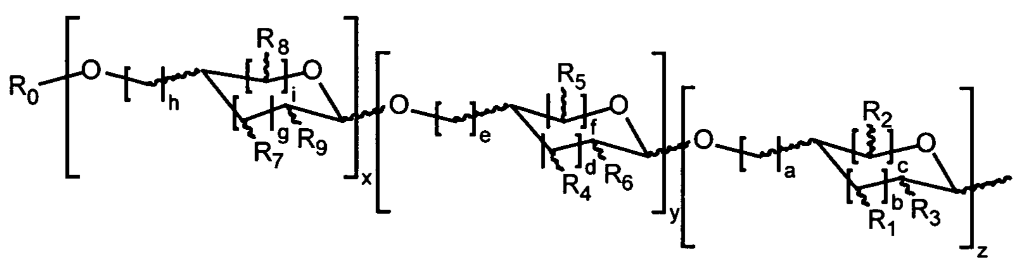
[0195] As discussed above, in one embodiment of the present invention, the inventive n-alkenyl glycosides can be conjugated either directly or through a crosslinker to an appropriate carrier (*e.g.*, KLH) to generate a synthetic tumor antigen. In general, a typical conjugation strategy that can be employed involves a reductive coupling of a glycoside which terminates in a glycoaldehyde, with the intended protein carrier, or lipid, presumably at the ε-amino acid

residues of exposed lysines. (M.A. Bernstein; L.D. Hall, *Carbohydr. Res.* **1980**, *78*, C1; R.V. Lemieux *Chem. Soc. Rev.* **1978**, *7*, 423).

[0196] Thus, in another aspect, the present invention provides synthetic constructs, whereby novel antigenic structures, as described herein, are conjugated to immunogenic carriers (*e.g.*, proteins, peptides or lipids). It will also be appreciated by one of ordinary skill in the art that, in the generation of a synthetic construct, more than one n-alkenyl moiety or glycopeptide moiety can ultimately be conjugated to a carrier protein to generate the synthetic vaccine. Thus, in addition to the conjugated glycopeptide structures as provided herein, constructs having the general structure as depicted below are also provided:

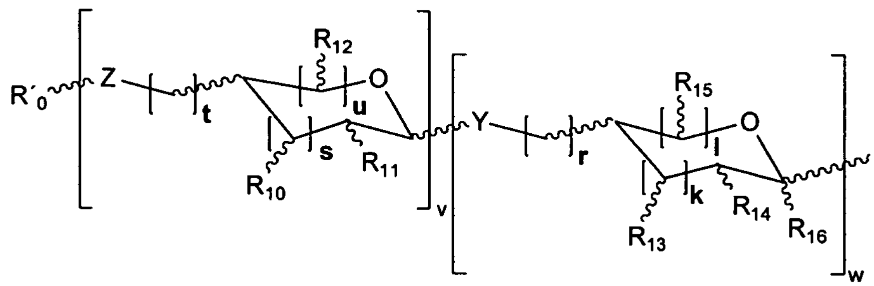


wherein A is a carbohydrate domain having the structure:



wherein a, b, c, d, e, f, g, h, i, x, y and z are independently 0, 1, 2 or 3, with the proviso that the x, y and z bracketed structures represent furanose or pyranose moieties and the sum of b and c is 1 or 2, the sum of d and f is 1 or 2, and the sum of g and i is 1 or 2, and with the proviso that x, y and z are not simultaneously 0; wherein R_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_1, R_2, R_3, R_4 , R_5, R_6 , R_7, R_8 and R_9 is independently hydrogen, OH, OR^i, NHR^i, $NHCOR^i$, F, CH_2OH, CH_2OR^i, a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i is independently

hydrogen, CHO, COOR^{ii}, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group or a saccharide moiety having the structure:



wherein Y and Z are independently NH or O; wherein k, l, r, s, t, u, v and w are each independently 0, 1 or 2; with the proviso that the v and w bracketed structures represent furanose or pyranose moieties and the sum of l and k is 1 or 2, and the sum of s and u is 1 or 2, and with the proviso that v and w are not simultaneously 0; wherein R'_0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R_{10}, R_{11}, R_{12}, R_{13}, R_{14} and R_{15} is independently hydrogen, OH, OR^{iii}, NHR^{iii}, NHCOR^{iii}, F, CH_2OH, CH_2OR^{iii}, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R_{16} is hydrogen, COOH, COOR^{ii}, CONHR^{ii}, a substituted or unsubstituted linear or branched chain alkyl or aryl group; wherein each occurrence of R^{iii} is hydrogen, CHO, COOR^{iv}, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group; and wherein each occurrence of R^{ii} and R^{iv} are each independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

wherein n is 0-8; wherein the carrier is an immunogenic carrier, wherein said carrier is linked directly or though a crosslinker; and wherein t′ is at least 1. In certain preferred embodiments, n is 4. In certain embodiments, the immunogenic carrier is a protein, peptide or lipid, including, but not limited to Human Serum Albumin, Bovine Serum Albumin, KLH and PamCys. In certain exemplary embodiments, t′ is in the range 20-600. In still other preferred embodiments, the carbohydrate determinant is selected from the group consisting of Globo-H, KH-1, glycophorin, STN, (2,3)ST, N3, Tn, TF, 2,6-STn, Gb3, Le^{y} and Le^{x}. In yet other preferred embodiments, the carbohydrate determinant is fucosyl GM1, which has the structure as

depicted above, and as shown in Figure 1. In certain embodiments, one or more of the alkylglycoside moieties is/are attached to the carrier via an –NH- moiety present on the carrier.

[0197] It will be appreciated that because certain of the inventive compounds produced terminate in an alkenyl linkage, in a typical protocol according to the present invention, conversion to an aldehyde is first required. Thus, in but one exemplary embodiment, an inventive synthetic globo-H tumor antigen is prepared from an n-alkenyl globo-H glycoside. As described in Example 2, this procedure involves exposing the n-alkenyl globo-H glycoside to oxidative conditions, in this case ozonolysis, followed by reductive work-up to yield an aldehyde intermediate to generate a vaccine glycoconjugate. Subsequent hydrolytic carbohydrate analysis reveals approximately 350 carbohydrate residues/molecule of carrier protein, as described in Example 2.

[0198] In yet another example, a fucosyl GM1-KLH glycoconjugate is generated according to the method of the present invention, as discussed in Example 1. Notably, prior to conjugation studies, synthetic n-pentenyl fucosyl GM1 was shown to bind to monoclonal antibody F12 in ELISA and immune thin layer chromatography assays. Inhibition studies revealed that preincubation of F12 with antibody completely inhibits reactivity of natural fucosyl GM1, with the antibody. Clearly, the synthetic fucosyl GM1 pentenyl glycoside provides the antigenic epitope with which F12 reacts on SCLC cells.

[0199] Additionally, once a synthetic vaccine has been derivatized and characterized, mouse immunological studies can be performed to assess the potency and/or specificity of the novel tumor vaccines, as described in Example 6 herein.

EQUIVALENTS

[0200] The representative examples which follow are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed, various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including the examples which follow and the references to the scientific and patent literature cited herein. In but one illustrative example, protecting groups play an important role in the synthesis of the carbohydrate domains and synthetic conjugates, as described herein; however it will be appreciated by one of ordinary skill

in the art that the present invention encompasses the use of various alternate protecting groups known in the art. Those protecting groups used in the disclosure including the Examples below are merely illustrative.

[0201] It should further be appreciated that the contents of those cited references are incorporated herein by reference to help illustrate the state of the art. The following examples contain important additional information, exemplification and guidance which can be adapted to the practice of this invention in its various embodiments and the equivalents thereof.

EXEMPLIFICATION

[0202] ***A. Example 1: Synthesis of fucosyl GM1 pentenyl glycoside***

[0203] ***1) Discussion of Synthesis:***

[0204] As discussed above, in one aspect of the invention, the synthesis of fucosyl GM1 pentenyl glycoside is provided. In one embodiment of the present invention, this was achieved similarly to the methodology employed in the synthesis of MBr1 antigen, Globo-H (see, Park *et al., J. Am. Chem. Soc.* **1996**, *118*, 11488). For example, as shown in Figure 2, the synthesis of the ABC trisaccharide starting from the known protected lactal derivative **2**, was first undertaken (Kwon, O.; Danishefsky. S.J. *J. Am. Chem. Soc.* **1998**, *120*, 1588). Selective sialylation of the C3' equatorial hydroxyl in **2** proceeded smoothly with phosphite donor **3** (Sim *et al.*, *J. Am. Chem. Soc.* **1993**, *115*, 2260; Chappell *et al.*, *Tetrahedron* **1997**, *53*, 11109) to yield the glycal **4** as the only observable isomer in 75% yield. In addition, propionitrile was employed as the solvent because of the necessity to perform the reaction at low temperatures. Use of elevated temperatures in acetonitrile as the solvent resulted in diminished anomeric selectivity, regioselectivity and lower chemical yields. The key DEF trisaccharide was synthesized as previously described in the Globo-H synthesis (Park *et al. J. Am. Chem. Soc.* **1996**, *118*, 11488). The requisite thioethyl donor **5** is shown in Figure 2. Based on previous experience, it was expected that this specific donor would favor β-glycosidation via sulfonamido participation under the close guidance of the "proximal hydroxyl" directing effect (see asterisk) (see also, Park *et al. J. Am. Chem. Soc.* **1996**, *118*, 11488; Kwon *et al. J. Am. Chem. Soc.* **1998**, *120*, 1588), and the results confirmed this expectation. In an experiment directed at "proof of principle", reaction of **5** with 5.0 equivalents of MeOTf (Lonn, H. *Carbo. Res.* **1985**, *134*, 105; Lonn, H. *J.*

Carbohydr. Chem. **1987**, *6*, 301) in the presence of **4** gave the desired hexasaccharide **6** in 23 % yield, as shown in Figure 3. Although direct deprotection of this compound was not achieved to yield the desired compound, in an effort to find a hexasaccharide which was suitable for global deprotection, replacement of the reducing end glycal was considered. Such a replacement would also be potentially useful as a linker capable of being modified to allow for conjugation to a protein carrier or lipid.

[0205] In but one example, the use of a n-pentenyl glycoside was considered (For a review of n-pentenyl glycosides, see Fraser-Reid *et al.*, *Synlett*, **1992**, 927; Udodong *et al. J. Am. Chem. Soc.* **1993**, *115*, 7886; Merritt *et al. J. Am. Chem. Soc.* **1994**, *116*, 8334; Fraser-Reid *et al.* **1990**, *55*, 6068; Mootoo *et al. J. Am. Chem. Soc.* **1988**, *110*, 2662; Mootoo *et al. J. Am. Chem. Soc.* **1989**, *111*, 8540 and references therein). N-pentenyl glycosides are stable to a range of reaction conditions and reagents, but are readily activated for glycosidation reactions by treatment with a halogen oxidant. As a result of their stability and the neutral conditions required for their activation, pentenyl glycosides have been demonstrated to be valuable linkages for mechanistic and synthetic studies. Additionally, a terminal pentenyl group, or more generally a terminal alkenyl group, could also provide a handle for bioconjugation. Thus, in one embodiment, glycal **6a** was subjected to epoxidation under standard procedures with 3,3-dimethyldioxirane (Figure 3). Reaction with pentenyl alcohol and anhydrous zinc chloride (Gordon *et al. Carbohydrate Res.* **1990**, *206*, 361) afforded the glycoside **7** in 65% yield. Indeed, with the pentenyl glycoside in place, global deprotection of **7** was possible. The sequence shown in Figure 3 furnished the peracetylated hexasaccharide lactone **8** in 46 % yield (5 steps). Removal of the acetates with sodium methoxide followed by saponification of the resulting methyl ester yielded the target, fucosyl GM1 pentenyl glycoside, **1b**. The assignment of structure **1b** was based on ^{1}H and ^{13}C NMR analysis of **1b**, in conjunction with characterization of intermediates en route to the final structure, and is supported by high resolution mass spectrometry.

[0206] In yet another embodiment, in an effort to produce significant quantities of this epitope for pre-clinical, and eventually clinical evaluation, a more efficient synthetic route was developed utilizing a glycoside at the reducing end at the acceptor, rather than a glycal. As shown in Figure 4, pentenyl lactoside was first investigated. For this purpose, lactose octaacetate was converted to the known bromide **9** (Reithal, Y. *J. Am. Chem. Soc.* 1952, 74, 4210; Dasgupta

et al. Carbohydr. Res. **1994**, *264*, 155). Reaction of this compound with pentenyl alcohol under promotion by silver carbonate delivered the desired pentenyl glycoside, **10**, on 100 g scale (Rodriguez, *et al. Aust. J. Chem.* **1990**, *43*, 665). An analogous coupling to produce **9** using silver triflate as promoter resulted in only a 17% yield of the desired product. Removal of the acetates yielded lactoside **11**. Again, the C3' and C4' hydroxyl groups were engaged, this time as the dimethyl ketal **12**. This reaction, as currently conducted, was accompanied by formation of minor amounts of 4,6-acetonide (Catelani *et al. Carb. Res.* **1988**, *182*, 297). Perbenzylation of **12** to give **13** followed by acetonide removal with aqueous acetic acid yielded the desired AB acceptor **14**. Sialylation using phosphite donor **3** (Figure 2) proceeded in comparable yield to give trisaccharide acceptor, **15**.

[0207] Finally, turning to the desired fucosyl GM1, coupling of donor **5** with a 2.0 molar excess of the acceptor **15** containing the pentenyl linker proceeded with MeOTf promotion (1.5 equivalents x 2) in excellent yield (70%; see Figure 5). Global deprotection under identical conditions as in Figure 4, yielded the characterized hexasaccharide **1b**.

[0208] Attention was then directed to the final goal of developing a glycoconjugate. Synthetic **1b** was subjected to conjugation to carrier protein KLH, as depicted in Figure 6. The protocol started with ozonolysis, thereby producing the uncharacterized aldehyde derivative. This step was followed by coupling to KLH using reductive amination under the agency of sodium cyanoborohydride. Presumably coupling of the carbohydrate had occurred with the ε-amino group of lysine residues in the KLH. Hydrolytic carbohydrate analysis revealed approximately 331 carbohydrate residues per molecule of KLH to generate **1c**.

[0209] *2) Experimentals*

[0210] **Peracetyl pentenyl-β-D-lactoside (10).** To a cooled (ice bath) suspension of lactose octaacetate (100.0 g, 147.7 mmol), glacial acetic acid (30 mL) and acetic anhydride (30 mL) was added 100 mL of 30% HBr in AcOH dropwise over a period of 60 minutes. The reaction mixture stirred for 1 hour and the ice bath was removed. Upon stirring for an additional 2 hours at room temperature, the reaction became a homogeneous yellow solution. The solution was diluted with H_2O (1000 mL) and extracted with $CHCl_3$ (3x400 mL). The organic extracts were washed with H_2O (2x1000 mL), satd. $NaHCO_3$ (3x500 mL), dried over $MgSO_4$ and concentrated. The α-bromo product **9** was azeotroped with anhydrous benzene and dried under

high vacuum to yield 98.8 g (96%) of the lactosyl bromide which was used without further purification.

[0211] To a suspension of Ag_2CO_3 (100 g, 362.6 mmol), freshly activated molecular sieves (15 g) and a crystal of I_2 in 400 mL CH_2Cl_2 was added pentenyl alcohol (5.0 equiv., 73.4 mL) and then the lactosyl bromide **9** (98.8 g, 141.4 mmol) in 400 mL of CH_2Cl_2. After stirring in the dark at room temperature for 16 hours, the reaction was filtered through a plug of celite with additional CH_2Cl_2 and concentrated to a yellow oil which was purified by flash column chromatography (10% EtOAc/hexanes->50% EtOAc/hexanes) to yield 74.7 g (75%) of the pentenyl lactoside **10** as a white foam. $[\alpha]^{22}_D$ –48.9° (*c* 7.5, $CHCl_3$); IR (film $CHCl_3$) 2941, 1751, 1369, 1224, 1054 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.60 (m, 1H), 5.17 (d, 1H, J=2.7 Hz), 5.02 (m, 1H), 4.93 (dd, 1H, J=7.9, 10.3 Hz), 4.85 (d, 1H, J=1.6 Hz), 4.78 (m, 2H), 4.71 (dd, 1H, J=9.6, 7.9 Hz), 4.30 (m, 3H), 3.93 (m, 3H), 3.66 (m, 3H), 3.45 (m, 1H), 3.30 (m, 1H), 1.98 (s, 3H), 1.94 (s, 3H), 1.91 (m, 2H), 1.89 (s, 3H), 1.88 (s, 6H, $2xCH_3$), 1.87 (s, 3H), 1.79 (s, 3H), 1.49 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.33, 170.28, 170.09, 170.00, 169.74, 69.54, 169.01, 137.72, 115.00, 101.01, 100.51, 76.27, 72.76, 72.48, 71.64, 70.94, 70.58, 69.23, 69.01, 66.52, 61.97, 60.73, 29.75, 28.49, 20.80, 20.75, 20.64, 20.57, 20.45; HRMS (FAB) cald. for $C_{31}H_{44}O_{18}Na$ $[M+Na]^+$ 727.2425, found 727.2418.

[0212] **Pent-4-enyl 3', 4'-*O*-acetonide-β-D-lactoside (12).** Peracteylated pentenyl lactoside, **10**, (18.2 g, 25.8 mmol) was dissolved in 300 mL of anhydrous MeOH and 2.0 mL of NaOMe (25% in MeOH) was added. The reaction stirred at rt for 16 hours and was neutralized with Dowex-H^+ (pH 5-6). The reaction was filtered with additional MeOH and concentrated to a white solid (10.6 g, quantitative) which was used without further purification. Pentenyl-β-D-lactoside **11**: ^1H NMR (D_2O, 400 MHz) δ 5.81 (m, 1H), 5.00 (dd, 1H, J=17.3, 1.9 Hz), 4.92 (dd, 1H, J=8.9 Hz), 4.74 (m, 1H), 4.39 (d, 1H, J=8.0 Hz), 4.35 (d, 1H, J=7.8 Hz), 3.72-3.42 (m, 12H), 3.21 (m, 1H0, 2.06 (m, 2H), 1.63 (m, 2H); ^{13}C NMR (D_2O, 100 MHz) δ 141.27, 117.31, 105.42, 104.54, 80.85, 77.84, 77.24, 76.92, 75.33, 75.00, 73.44, 72.47, 71.03, 63.52, 62.56, 31.83, 30.48.

[0213] To pentenyl lactoside **11** (10.6 g, 25.8 mmol) was added 200 mL acetone, 26 mL of dimethoxypropane and *p*-toluenesulfonic acid (491 mg, 0.1 equiv.). The suspension stirred at

room temperature overnight at which point the reaction was homogeneous. The reaction was concentrated and purified by flash column chromatography (100% EtOAc -> 2%MeOH in EtOAc) to give 5.1 g (44%) of the 3,4-acetonide as a white solid and 1.27 g of the 4,6-acetonide as a white solid. **3,4-acetonide, 12:** $[\alpha]^{22}_D$ 79.0° (0.96*c* , MeOH); IR 3422, 2980, 2933, 2870, 1242, 1073 cm^{-1}; ^1H NMR (MeOH, 400 MHz) δ 5.83 (m, 1H), 5.00 (dd, 1H, J=17.1, 3.4 Hz), 4.92 (dd, 1H, J=10.2, 2.0 Hz), 4.34 (d, 1H, J=8.2 Hz), 4.25 (d, 1H, J=7.8 Hz), 4.17 (dd, 1H, J=5.5, 2.1 Hz), 4.02 (dd, 1H, J=7.2, 5.5 Hz), 3.91 (m, 3H), 3.88-3.73 (m, 5H), 3.55-3.47 (m, 3H), 3.42 (m, 1H), 3.38 (m, 1H), 3.21 (m, 1H), 2.13 (m, 2H), 1.67 (m, 2H), 1.45 (s, 3H), 1.30 (m, 3H); ^{13}C NMR (MeOH, 100 MHz) δ 139.42, 115.20, 111.04 (O-C-O), 104.16, 104.09, 80.94, 80.77, 76.29, 76.25, 75.27, 75.00, 74.76, 74.39, 62.36, 61.82, 31.18 (CH_3), 30.02, 28.41, 26.51 (CH_3); HRMS (Fab) cald. for $C_{20}H_{34}O_{11}Na$ $[M+Na]^+$ 473.1998, found 473.1985. **4,6-acetonide:** $[\alpha]^{22}_D$ -216.0 ° (*c* 1.14, MeOH); IR 3364, 2926, 2870, 1380 cm^{-1}; ^1H NMR (MeOH, 400 MHz) δ 5.79 (m, 1H), 4.98 (dd, 1H, J=17.0, 1.8), 4.90 (dd, 1H, J=10.2, 1.0), 4.35 (d, 1H, J-7.8 Hz), 4.24 (d, 1H, J=7.8 Hz), 4.13 (m, 2H), 3.86-3.82 (m, 3H), 3.76 (dd, 1H, J=12.9, 1.4 Hz), 3.61-3.49 (m, 5H), 3.44 (s, 1H), 3.35 (m, 1H), 3.19 (t, 1H), 2.11 (m, 2H), 1.66 (m, 2H), 1.43 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (MeOH, 100 MHz) δ 139.48, 115.20, 104.68, 104.26, 100.17 (O-C-O), 79.86, 76.40, 76.35, 74.81, 73.34, 71.46 ,70.25, 69.95, 68.04, 63.67, 61.69, 31.23 (CH_3), 30.08, 29.56, 18.69 (CH_3).

[0214] **Pent-4-enyl 2, 3, 6, 2', 6'-penta-*O*-benzyl-3', 4'-*O*-acetonide-β-D-lactoside (13).** The acetonide **12** (3.40 g, 7.54 mmol) was azeotroped with anhydrous benzene, dissolved in anhydrous DMF (60 mL, 0.12 M) and cooled to 0 °C. Benzyl bromide (passed through basic alumina, 10.0 equiv. 8.97 mL), was added followed by solid NaH (95%, 7.5 equiv., 1.76 g) in one portion. The reaction was allowed to warm to room temperature overnight and then poured into ice cold H_2O (500 mL) and extracted with $CHCl_3$ (200 mL, 2x100mL). The organic extracts were washed with brine (500 mL), dried over $MgSO_4$ and concentrated to a yellow oil which was purified by flash column chromatography (5% EtOAc/hexanes -> 20% EtOAc/hexanes) to yield 5.70 g (84%) of the product as a viscous oil. $[\alpha]^{22}_D$ 196.0° (1.09*c* , $CHCl_3$); IR (film $CHCl_3$) 3062, 3029, 2868, 1367, 1093, 1055 cm^{-1}; ^1H NMR ($CDCl_3$, 400

MHz) δ 7.39-7.20 (m, 25H), 5.78 (m, 1H), 4.98 (dd, 1H, J=17.1, 3.4 Hz), 4.93 (dd, 1H, J=10.2, 2.0 Hz), 4.89 (d, 1H, J=10.6 Hz), 4.86 (d, 1H, J=10.9 Hz), 4.75 (d, 1H, J=11.7 Hz), 4.70 (d, 1H, J=10.6 Hz), 4.68 (d, 1H, J=10.8 Hz), 4.63 (d, 1H, 11.8 Hz), 4.53 (d, 1H, J=12.1 Hz), 4.46 (d, 1H, J=12.1 Hz), 4.39 (d, 1H, J=6.3 Hz), 4.36 (d, 1H, J=2.0 Hz), 4.34 (d, 1H, J=7.8 Hz), 4.28 (d, 1H, J=12.0 Hz), 4.07 (dd, 1H, J=5.5, 1.4 Hz), 3.99 (m, 1H), 3.91 (m, 2H), 3.76 (dd, 1H, J=11.9, 4.2 Hz), 3.70 (dd, 1H, J=10.8, 1.6 Hz), 3.65 (m, 2H), 3.55-3.47 (m, 3H), 3.35 (m, 2H), 3.30 (dd, 1H, J=7.9, 0.8 Hz), 2.31 (m, 2H), 1.72 (m, 2H), 1.36 (s, 3H), 1.31 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.92, 138.54, 138.43, 138.31, 138.19, 138.00, 128.22, 128.16, 128.08, 127.99, 127.95, 127.77, 127.63, 127.49, 127.40, 127.34, 127.20, 114.83, 109.66, 103.56, 101.76, 82.89, 81.75, 80.53, 79.26, 76.30, 75.32, 74.96, 74.91, 73.51, 73.28, 73.12, 73.08, 71.86, 69.16, 68.82, 68.18, 30.15, 28.87, 27.89, 26.34; HRMS (FAB) cald. for $[M+Na]^+$ $C_{55}H_{64}O_{11}Na$ 923.4346, found 923.4330.

[0215] **Pent-4-enyl 2, 3, 6, 2', 6'-penta-*O*-benzyl-β-D-lactoside (14).** The acetonide **13** (5.7 g, 6.32 mmol) was dissolved in 80% AcOH in H_2O (60 mL) and heated to 75 °C for 3 hours. The reaction was cooled to rt, diluted with H_2O (500 mL) and extracted with $CHCl_3$ (200 mL, 2x100 mL). The organic extracts were washed with H_2O (500 mL), satd. $NaHCO_3$ (3x300 mL), dried over $MgSO_4$ and concentrated to an oil which was purified by flash column chromatography (25% EtOAc/hexanes) to yield 5.21 g (96%) of a white solid. $[\alpha]^{22}_D$ 194.1° (1.13*c* , $CHCl_3$); IR (film $CHCl_3$) 3444, 3028, 2868, 1091, 1058 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.41-7.20 (m, 25H), 5.74 (m, 1H), 4.96-4.88 (m, 3H), 4.82 (d, 1H, J=10.9 Hz), 4.72 (d, 1H, J=11.4 Hz), 4.70 (d, 1H, J=10.9 Hz), 4.64 (d, 1H, J=10.9 Hz), 4.58 (d, 1H, J=11.6 Hz), 4.52 (d, 1H, J=10.9), 4.38-4.28 (m, 5H), 3.93-3.85 (m, 3H), 3.71 (m, 2H), 3.55-3.40 (m, 4H), 3.36 (m, 3H), 3.28 (m, 2H), 2.48 (d, OH, 1H, J=3.2 Hz), 2.40 (s, OH, 1H), 2.09 (m, 2H), 1.66 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.09, 138.53, 139.29, 138.17, 138.01, 137.91, 128.44, 128.35, 128.24, 127.89, 127.82, 127.77, 127.63, 127.55, 127.19, 114.86, 103.57, 102.53, 82.76, 81.71, 79.96, 76.57, 75.17, 75.02, 74.89, 74.80, 73.45, 73.40, 73.12, 72.79, 69.22, 68.71, 68.60, 68.24, 30.17, 28.88; HRMS (FAB) cald. for $C_{52}H_{60}O_{11}Na$ $[M+Na]^+$883.4033, found 883.4017.

[0216] **Trisaccharide 15**. The phosphite donor **3** (1.0 g, 1.35 mmmol) and lactosyl acceptor **14** (2.5 g, 2.90 mmol) were combined, azeotroped with anhydrous benzene and placed

under high vacuum for 2 hours. The mixture was dissolved in anhydrous CH_3CH_2CN (distilled from CaH_2), freshly activated molecular sieves were added and the reaction cooled to -40 °C. A portion of TMSOTf (0.1 equiv., 27μL) was added and the reaction was allowed to stir at -40 °C overnight. The reaction was then warmed to -30 °C and another 0.1 equivalent of TMSOTf was added. Upon stirring for an additional 2 hours at -30 °C, the reaction was quenched by the addition of solid $NaHCO_3$ and was filtered through a plug of celite with the aid of EtOAc. The organic layer was washed with satd. $NaHCO_3$ (2x400 mL) and dried over $MgSO_4$. Evaporation of organic layer gave a cloudy oil which was subjected to flash column chromatography using careful gradient elution in order to recover acceptor and product trisaccharide (20% EtOAc/hexanes -> 75% EtOAc/hexanes). The product (1.35g, 75%) was obtained as a white foam and 0.95 g of starting acceptor was recovered. : $[\alpha]^{22}{}_D$ 2.38° (*c* 1.30, $CHCl_3$); IR (film $CHCl_3$) 3106, 2866, 1744, 1689, 1368, 1222, 1055 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.40-7.17 (m, 25H), 5.79 (m, 1H), 5.38 (m, 1H), 5.27 (dd, 1H, J=8.0, 2.0 Hz), 5.08 (d, 1H, J=10.0 Hz), 4.95 (m, 3H), 4.86 (d, 1H, J=10.9 Hz), 4.75 (d, 1H, J=5.7 Hz), 4.72 (d, 1H, J=10.8 Hz), 4.68 (d, 1H, J=11.0 Hz), 4.56 (d, 1H, J=11.9 Hz), 4.54 (d, 1H, J=7.6 Hz), 4.44 (d, 1H, J=12.2 Hz), 4.39 (m, 1H), 4.32-4.25 (m, 3H), 4.06-3.88 (m, 6H), 3.79 (m, 2H), 3.72 (s, 3H), 3.65 (m, 3H), 3.54-3.44 (m, 5H), 3.35 (m, 2H), 2.66 (d, OH, 1H, J=3.3 Hz), 2.47 (dd, 1H, J=13.0, 4.7 Hz), 2.12 (m, 2H), 2.06 (s, 3H), 2.02 (m, 1H), 1.98 (s, 3H), 1.95 (s, 3H),1.85 (s, 3H), 1.83 (s, 3H), 1.71 (s, 3H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.77, 170.53, 170.23, 169.92, 169.87, 168.32, 139.09, 138.90, 138.61, 138.45, 138.34, 138.05, 128.27, 128.21, 137.99, 127.51, 127.42, 127.11, 114.81, 103.48, 102.29, 98.32, 82.90, 81.80, 78.37, 76.50, 76.30, 75.31, 75.01, 74.89, 74.82, 73.23, 72.97, 72.66, 72.37, 69.16, 69.03, 68.69, 68.43, 68.36, 67.81, 67.08, 62.21, 52.99, 49.17, 36.41, 30.17, 28.89, 23.11, 21.08, 20.77, 60.67, 60.47; HRMS (FAB) cald. for $C_{72}H_{87}NO_{23}Na$ ($M+Na^+$) 1356.5566, found 1356.5557.

[0217] **Hexasaccharide 7 (R=Bn).** The thioethyl donor **5** (311 mg, 0.243 mmol) and acceptor **15** (627 mg, 0.487 mmol) were combined, azeotroped with anhydrous benzene (5x5 mL) and placed under high vacuum for 5 hours. The mixture was then dissolved in 1.6 mL CH_2Cl_2 and 3.2 mL Et_2O (0.05M total), treated with freshly prepared molecular sieves and cooled to 0 °C. Methyl triflate (1.5 equiv., 41 μL) was added in one portion and the reaction

stirred at 0 °C overnight. In the morning, another 20 μL of MeOTf was added and the reaction was allowed to stir for an additional 2 hours at 5 °C. The reaction was quenched by the addition of solid $NaHCO_3$, filtered through celite with EtOAc, concentrated and purified by flash column chromatography (gradient elution 25% EtOAc/hexanes -> 50% -> 75% EtOAc/hexanes) to give 437 mg (70%) of hexasaccharide as a white foam and 300 mg of recovered trisaccharide accpetor: $[\alpha]^{22}_D$ -29.4° (*c* 3.25, $CHCl_3$); IR (film $CHCl_3$) 3285, 3028, 2940, 2865, 1794, 1749, 1690, 1220, 1090 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.74 (d, 2H, J=7.5 Hz), 7.34-7.08 (m, 43H), 5.75 (m, 1H), 5.52 (d, 1H, J=4.7 Hz), 5.29 (app s, 1H), 5.23 (dd, 1H, J=9.5, 1.4 Hz), 5.15 (m, 1H), 5.02 (d, 1H, J=9.8 Hz)4.97-4.87 (m, 5H), 4.84 (d, 1H, J=10.9 Hz), 4.81-4.70 (m, 5H), 4.63 (d, 1H, J=11.6 Hz), 4.57 (m, 3H), 4.44 (d, 1H, J=7.2 Hz), 4.40 (d, 1H, J=12.2 Hz), 4.30 (d, 1H, J=7.8 Hz), 4.10 (m, 2H), 3.98-3.81 (m, 12H), 3.82 (s, 3H), 3.78-3.68 (m, 7H), 3.64-3.45 (m, 8H), 3.27 (m, 3H), 3.17 (dd, 1H), 2.80 (d, OH, 1H, J=2.1 Hz), 2.19 (dd, 1H, J=13.0, 4.5 Hz), 2.10 (m, 3H), 2.01 (s, 3H), 1.92 (s, 3H), 1.88 (s, 3H), 1.82 (s, 3H), 1.81 (s, 3H), 1.68 (m, 2H), 1.08 (d, 3H, J=5.4 Hz), 1.00-0.92 (m, 42H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.61, 170.34, 170.26, 169.66, 167.78, 155.48, 138.95, 138.65, 138.63, 138.56, 138.42, 138.38, 138.27, 138.05, 132.17, 129.02, 128.59, 128.46, 128.18, 128.05, 127.91, 127.63, 127.51, 127.24, 127.09, 114.80, 103.42, 102.76, 102.45, 100.16, 99.58, 98.76, 82.87, 81.53, 79.06, 77.32, 77.24, 77.16, 75.12, 75.07, 74.95, 74.80, 73.92, 73.27, 73.04, 72.93, 72.19, 69.23, 69.14, 69.09, 67.89, 67.53, 61.76, 61.58, 61.12, 56.39, 53.60, 49.19, 35.36, 30.17, 28.89, 23.13, 20.97, 20.75, 20:62, 20.53, 17.85, 17.53, 17.33, 16.72, 11.80, 11.74; HRMS (FAB) cald. for $C_{136}H_{178}N_2O_{39}SSi_2$ (M+Na^+) 2574.1163, found 2574.1130.

[0218] **Compound 1b.** To a solution of the hexasaccharide (130 mg, 0.0509 mmol) in THF (2.0 mL) was added glacial AcOH (10.0 equiv., 29 μL) and TBAF (1.0 M THF, 10.0 equiv., 0.509 mL). The reaction stirred at rt overnight, was poured into ice water and extracted with EtOAc (3x50 mL). The organic extracts were washed with satd. $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated to an oil which was purified through a short plug of silica gel with EtOAc. The resulting triol was dissolved in anhydrous MeOH (2.5 mL) and sodium methoxide was added (0.250 mL of a 25% solution in MeOH). The reaction stirred at rt for 18 hours and then 0.5 mL of THF and 0.5 mL of H_2O were added. Stirring at rt for an

additional 24 hours was followed by neutralization with Dowex-H^+, filtration with MeOH washings and concentration. The crude material was allowed to dry under high vacuum for 1 day. To the resulting white solid was added THF (0.5 mL) and condensed liquid NH_3 (~10 mL) at -78 °C. Sodium (~50 mg) was added and the resulting blue solution stirred at -78 °C for 1.5 hours. The reaction was quenched with anhydrous MeOH (~5 mL), brought to rt and concentrated with a stream of dry N_2 to a volume of ~ 2 mL. The reaction was neutralized with Dowex-H^+, filtered with MeOH washings and concnetrated to a white solid. The white solid was dissolved in 1.0 mL pyridine and 1.0 mL CH_2Cl_2 and cooled to 0 °C. A crystal of DMAP was added followed by acetic anhydride (1.0 mL). The ice bath was removed and the reaction stirred at rt overnight. Concentration followed by purification by flash column chromatography (gradient elution 75% EtOAc/hexanes -> 100% EtOAc -:> 5% MeOH/EtOAc) gave 44 mg (46%) of **8** as a white solid: ^1H NMR (MeOH, 400 MHz) δ 8.02 (d, 1H, J=9.9 Hz), 7.87 (d, 1H, J=9.2 Hz), 5.76 (m, 1H), 5.49 (m, 1H), 5.39 (d, 1H, J=2.9 Hz), 5.34-5.31 (m, 2H), 5.22 (d, 1H, J=3.4 Hz), 5.19 (d, 1H, J=4.1 Hz), 5.17 (d, 1H, J=3.5 Hz), 5.12-5.05 (m, 3H), 4.97 (dd, 1H, J=16.8, 1.7 Hz), 4.91 (dd, 1H, J=10.0, 1.7 Hz), 4.81-4.75 (m, 3H), 4.65-4.60 (m, 2H), 4.52 (d, 1H, J=7.9 Hz), 4.48-4.44 (m, 2H), 4.37 (dd, 1H, J=10.0, 2.5 Hz), 4.28 (dd, 1H, J=12.5, 2.4 Hz), 4.22-4.18 (m, 2H), 4.14-3.99 (m, 9H), 3.96-3.92 (m, 2H), 3.89 (d, 1H, J=2.9 Hz), 3.88-3.77 (m, 4H), 3.72-3.62 (m, 3H), 3.51-3.45 (m, 1H), 2.74 (dd, 1H, J=11.3, 4.5 Hz), 2.19 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), .91 (s, 3H), .180 (s, 3H), 1.61 (m, 2H), 1.14 (d, 3H, J=6.4 Hz), 3 protons burried beneath acetates (2 Pn, 1 C3ax); ^{13}C NMR (MeOH, 100 MHz) δ 174.64, 173.64, 172.98, 172.89, 172.63, 172.56, 172.48, 172.44, 172.34, 172.27, 172.04, 171.99, 171.76, 171.73, 171.62, 171.35, 171.25, 139.23, 115.47, 104.62, 103.26, 101.86, 101.63, 100.78, 97.31, 78.22, 76.53, 75.08, 74.69, 74.29, 73.91, 73.53, 72.94, 72.71, 72.56, 72.16, 72.06, 71.89, 71.74, 70.19, 69.87, 69.33, 69.11, 68.92, 65.96, 65.65, 63.68, 63.52, 62.69, 54.01, 53.09, 50.60, 40.19, 31.09, 29.96, 24.17, 24.06, 22.73, 21.76, 21.59, 21.46, 21.20, 21.06, 20.89, 20.75, 20.63, 20.55, 16.52.

[0219] The peracetate (40 mg) was dissolved in anhydrous MeOH (2.0 mL) and 150 μL of sodium methoxide was added (25% solution in MeOH). The reaction stirred at rt for 18 hours

and then 0.5 mL of THF and 0.5 mL of H_2O was added. The reaction stirred for another 24 hours at rt. Neutralization with Dowex-H^+ (~pH 6-7) was followed by filtration with MeOH washings, concentration and purification using P-2 Gel (H_2O elutent) to yield 24 mg (96%) of a white solid: IR 3346, 2940, 2882, 1657, 1620, 1376, 1069 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 5.86 (m, 1H), 5.18 (d, 1H, J=4.0 Hz), 5.04 (dd, 1H, J=17.22, 1.7 Hz), 4.97 (dd, 1H, J=10.6 Hz), 4.63 (d, 1H, J=7.6 Hz), 4.57 (d, 1H, J=7.7), 4.46 (d, 1H, J=7.9 Hz), 4.43 (d, 1H, J=8.1 Hz), 4.15 (m, 1H), 4.09-4.02 (m, 3H), 3.94-3.84 (m, 5H), 3.80-3.63 (m, 18H), 3.60-3.53 (m, 6H), 3.47 (dd, 1H, J=10.3, 1.8), 3.32 (t, 1H), 3.26 (t, 2H), 2.62 (dd, 1H, J=13.4, 4.3 Hz), 2.09 (m, 2H), 1.98 (s, 6H), 1.86 (m, 1H), 1.67 (m, 2H), 1.15 (d, 3H, J=6.5 Hz) ; ^{13}C NMR (D_2O, 100 MHz) δ 176.29, 175.43, 175.16, 139.97, 115.99, 104.38, 103.77, 103.30, 103.22, 102.25, 100.35, 79.67, 78.12, 77.65, 77.03, 76.06, 75.94, 75.62, 75.44, 75.24, 74.85, 74.19, 74.01, 73.45, 73.01, 71.15, 70.72, 70.32, 69.87, 69.64, 69.25, 67.93, 64.01, 62.29, 62.07, 61.63, 61.29, 52.79, 52.70, 50.04, 38.45, 30.53, 29.17, 23.89, 23.23, 16.53; HRMS (FAB) cald. for $C_{48}H_{79}N_2O_{33}Na_2$ [M-H+2Na]$^+$ 1257.4360, found 1257.4337.

[0220] **Glycal hexasaccharide 6a**. The thioethyl donor **5** (120 mg, 0.0938 mmol) and acceptor **4** (122 mg, 0.108 mmol) were combined, azeotroped with anhydrous benzene (5x5mL) and placed under high vacuum overnight. The mixture was dissloved in a 2:1 mixture of Et_2O:CH_2Cl_2 (2.7 mL total), molecular sieves were added and the mixture stirred at rt for 1 h. The reaction was cooled to 0 °C and 1.0 equiv. of MeOTf (0.020 mL) was added. After 4 hours at 0 °C another equivalent of MeOTf was added (0.020mL) and the reaction continued to stir for another 4 h at 10 °C. The reaction was quenched with solid $NaHCO_3$, filtered through celite with additional EtOAc (100ml) and concentrated. The resulting mixture was purified by flash column chromatography to give 50 mg (23%) of the hexasaccharide glycal **6** and 85 mg of starting acceptor, **4**: R_f 0.35 (66% EtOAc/hexanes); ^1H NMR (500 MHz, C_6D_6) δ 8.31 (d, 2H), 7.62 (d, 2H), 7.52 (m, 4H), 7.45 (d, 2H), 7.40-7.15 (m, 31H), 6.47 (d, 1H, J=6.3 Hz), 6.28 (apparent s, 1H), 6.09 (d, 1H, J=3.8 Hz), 5.72 (m, 1H), 5.55 (dd, 1H, J=9.3, 1.2 Hz), 5.51 (d, 1H, J=3.5 Hz), 5.22 (d, 1H, J=10.8 Hz), 5.15 (s, 1H), 5.13-5.06 (m, 3H), 5.05 (d, 1H, J=8.1 Hz), 5.02 (m, 1H), 4.98 (d, 1H, J=10.8 Hz), 4.85 (d, 1H, J=10.6 Hz), 4.82 (d, 1H, J=9.4 Hz), 4.73-4.66 (m, 8H), 4.55-4.34 (m, 10H), 4.38-4.32 (m, 5H), 4.30 (d, 1H), 4.18 (s, 3H), 4.21-4.12 (m, 6H), 4.06

(m, 2H), 3.99 (m, 4H), 3.85 (d, 1H), 3.74 (dd, 1H), 3.61 (m, 2H), 3.52 (t, 1H), 2.63 (dd, 1H, J=13.9, 5.0), 2.48 (dd, 1H, J=13.4 Hz), 2.35 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.72 (s, 3H), 1.64 (s, 3H), 1.57 (d, 3H, J=6.3), 1.31-1.20 (m, 42H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.71, 169.39, 169.18, 168.70, 168.12, 166.99, 154.75, 143.47, 137.81, 137.71, 137.51, 137.42, 137.07, 131.65, 128.25, 127.52, 128.32, 127.26, 127.23, 127.19, 127.10, 126.98, 126.91, 126.83, 126.73, 126.62, 126.53, 126.36, 126.29, 101.67, 101.35, 98.69, 98.32, 98.26, 97.33, 80.48, 78.05, 77.06, 76.20, 75.50, 74.64, 74.22, 73.87, 73.49, 72.90, 72.38, 72.26, 71.93, 71.47, 71.20, 70.34, 70.17, 69.99, 69.13, 68.62, 68.10, 67.92, 67.01, 66.88, 66.68, 65.52, 60.92, 60.61, 55.51, 52.59, 48.31, 34.87, 28.68, 22.19, 19.95, 19.77, 19.68, 19.59, 16.93, 16.88, 15.79, 10.86, 10.78; HRMS (FAB) cald. for $C_{124}H_{162}N_2O_{37}Si_2SNa$ $[M+Na]^+$ 2382.0013, found 2382.0001.

[0221] **Imido-hexasaccharide 6b** Performing the above reaction with 10 equiv. MeOTf added in one portion, under otherwise identical conditions yields 28% of the following compound, which is much less polar than the parent N-acetylated hexasaccharide **6a**. R_f 0.35 (25% EtOAc/hexanes); ^1H NMR (500 MHz, C_6D_6) δ 8.31 (d, 2H), 7.66 (d, 2H), 7.53 (t, 4H), 7.48 (d, 2H), 7.42-7.16 (m, 31H), 6.46 (d, 1H), 6.21 (app s, 1H), 6.15 (d, 1H, J=4.3 Hz), 5.81 (d, 1H, J=9.2 Hz), 5.72 (dt, 1H, J=12.8, 2.4 Hz), 5.40 (m, 1H), 5.38 (d, 1H, J=3.5 Hz), 5.20 (d, 1H, J=10.2 Hz), 5.12 (t, 2H), 5.00 (m, 3H), 4.84 (d, 1H, J-6.2 Hz), 4.81 (d, 1H, J=4.5 Hz), 4.73 (m, 2H), 4.70 (m, 2H), 4.67 (d, 1H, J=2.6 Hz), 4.65 (m, 1H), 4.59 (m, 3H), 4.53-4.46 (m, 6H), 4.40 (m, 5H), 4.36 (d, 1H, J=3.1 Hz), 4.30 (d, 1H, J=3.4 Hz), 4.26 (m, 3H), 4.23 (app s, 1H), 4.20 (m, 3H), 4.11 (m, 2H), 4.04 (d, 1H, J=5.9 Hz), 3.99 (s, 3H), 3.92 (d, 1H, J=3.2 Hz), 3.87 (d, 1H, J=2.9 Hz), 3.82 (d, 1H, J=6.5 Hz), 3.70 (m, 1H), 3.64 (s, 3H), 3.60 (d, 1H), 3.28 (t, 1H), 2.94 (dd, 1H, J=13.7, 4.5 Hz), 2.36 (t, 1H, J=13.3 Hz), 2.14 (s, 3H), 1.91 (s, 3H), 1.83 (s, 3H), 1.81 (s, 3H), 1.60 (s, 3H), 1.53 (d, 3H, J=6.5 Hz), 1.32-1.23 (m, 42H); ^{13}C NMR (100 MHz, $CHCl_3$) δ 170.43, 169.30, 169.20, 168.98, 168.03, 164.74, 155.82, 144.74, 139.09, 138.75, 138.52, 138.48, 138.40, 138.39, 138.25, 138.17, 132.56, 129.22, 128.85, 128.39, 128.35, 128.30, 128.25, 128.01, 127.79, 127.71, 127.60, 127.55, 127.50, 127.48, 127.34, 102.57, 102.24, 99.69, 99.11, 98.25, 81.35, 79.09, 87.22, 75.64, 75.40, 74.90, 74.60, 74.15, 73.95, 73.50, 73.33, 72.94, 72.84, 72.52, 71.37, 71.17, 70.47, 70.17, 69.66, 69.05, 68.47, 68.11, 67.96, 67.71, 67.55, 61.91, 61.54, 61.05, 57.70, 56.50, 53.65, 52.75, 31.94, 29.71, 21.70, 20.97, 20.89, 20.64, 20.46, 20.44, 17.57, 16.81, 15.38, 14.13, 11.89, 11.80; LRMS (FAB) $C_{125}H_{164}N_2O_{37}SSi_2Na$ 2373 $[M+Na]^+$.

[0222] *3) Conjugation studies:*

[0223] As described herein and as shown in Figure 6, the pentenyl group in FucGM1 was converted to an aldehyde group by ozonolysis and linked to -NH_2 groups of KLH by reductive amination method in the presence of sodium cyanoborohydride as described for globo H (see, Ragupathi G, Park TK, Zhang S, Kim IJ, Graeber L, Adluri S, Lloyd KO, Danishefsky SJ and Livingston PO. Immunization of mice with conjugates of fully synthetic hexasaccharide globo H results in antibody against human cancer cells: a combined chemical immunological approach to the fashioning of an anticancer vaccine. *Angewandte Chem. Int. Ed Engl. 36: 125-128. 1997.*). In the case of cross-linker method the aldehyde group obtained through ozonolysis was first reacted with hydrazide of MMCCH (4-(maleimidomethyl) cyclohexane-1-carboxyl hydrazide) and reacted with thiolated KLH as described in Ragupathi G, Koganty RR, Qiu D, Lloyd KO and Livingston PO. A novel and efficient method for synthetic carbohydrate conjugate vaccine preparation: Synthesis of sialyl Tn-KLH conjugate using a 4-(4-N-maleimidomethyl) cyclohexane-1-carboxyl hydrazide (MMCCH) linker arm. *Glycoconjugate J.*, **15**: 217-221, 1998). For example, 4 mg of FucGM1 pentenyl glycoside in methanol was stirred at -78°C in a dry-ice/ethanol bath and ozone gas was passed through the solution for 10 min under vigorous stirring. The excess of ozone was then displaced with nitrogen over a period of 5 min. Methyl sulfide (100 μl) was added and the reaction mixture stirred at room temperature for 2 hours and distributed equally in two vials. The solvent was removed under a stream of nitrogen. The resulting white solid was used directly in the subsequent conjugation steps.

[0224] ***a) Direct Conjugation of FucGM1-aldehyde with KLH:***

[0225] Two mg FucGM1-aldehyde was dissolved in 1 ml of 0.1M phosphate buffered saline (PBS) pH 7.2 and 4 mg of KLH in PBS. Two mg sodium cyanoborohydride was added and the mixture incubated under gentle agitation at 37°C for 48 h. After 16 h, an additional 1.0 mg sodium cyanoborohydride was added and the incubation continued. The unreacted FucGM1 aldehyde was removed completely with multiple washes using a Amicon Centriprep with molecular weight cut-off value 30000 dalton, with 6-7 changes of PBS at 4°C.

[0226] ***b) Conjugation of FucGM1-aldehyde through MMCCH to thiolated KLH:***

[0227] *Preparation of FucGM1-MMCCH*

[0228] Two mg of FucGM1-aldehyde was dissolved in 1 ml of 0.1M sodium acetate buffer pH 5.5, and 4 mg of MMCCH in 100 µl of dimethyl sulfoxide (DMSO) was added. The reaction mixture was incubated at room temperature for 15 min with gentle stirring. At the end of 15 min 2 mg of solid sodium cyanoborohydride was added and the incubation continued at room temperature for 2 h. Unreacted MMCCH was removed in a Sephadex G10 column equilibrated previously with 0.1 M sodium phosphate buffer pH 6.0 containing 5 mM EDTA and eluted with the same buffer. The fractions positive for FucGM1 by TLC with orcinol were combined.

[0229] *Addition of sulfhydryl groups to KLH*

[0230] 2-Iminothiolane (2 mg) dissolved in thiolation buffer (50 mM triethanolamine, 0.15 M NaCl, 5 mM EDTA, pH 8.0) was added to 4 mg of KLH and incubated with stirring at room temperature for 2 h. Unreacted 2-iminothiolane was removed by Sephadex G15 column equilibrated previously with 0.1 M sodium phosphate buffer pH 7.2 containing 5 mM EDTA and eluted with the same buffer. Fractions positive for KLH with BioRad protein assay dye reagent were combined. A small portion was used to estimate sulfhydryl groups in the thiolated KLH using Ellman's reagents and cysteine as standard as described earlier (Riddles PW, Blackeley RL, Zerner B Ellman's reagent: 5,5'-dithiobis(2-nitrobenzoic acid)--a reexamination, *Anal Biochem* **94**: 75-81, 1979). The KLH was estimated by a dye method using BioRad dye reagent according to the manufacture's instructions.

[0231] *Conjugation of FucGM1-MMCCH to thiolated KLH*

[0232] The FucGM1-MMCCH product and thiolated KLH were mixed and adjusted to pH 7.2 with 0.1M sodium phosphate buffer pH 8.0. The reaction mixture was then incubated at room temperature overnight. The content of the FucGM1-MMCCH-KLH reaction vial was transferred to a Centriprep concentrator 30 (Amicon: molecular cut-off 30000 Dalton) and unreacted FucGM1-MMCCH was removed completely with multiple washes. The conjugate was checked by HPTLC for the absence of unreacted FucGM1 as mentioned above. The epitope ratios of two batches of conjugate were determined by estimating protein content by BioRad dye binding protein assay and carbohydrate by a HPAEC-PAD assay. The epitope ratio of FucGM1-KLH (by direct method) and FucGM1-MMCCH-KLH was 149/1 and 1527/1 respectively.

[0233] *B. Example 2: Synthesis of Globo-H and conjugates thereof:*

[0234] *1) Discussion of Synthesis:*

[0235] In yet another embodiment of the present invention, an improved synthesis of Globo-H is provided utilizing the novel synthetic methodology as presented herein. The previous synthesis of globo-H described by the present inventors (Park *et al. J. Am. Chem. Soc.* **1996**, *118*, 11488; Bilodeau *et al. J. Am. Chem. Soc.* **1995**, *117*, 7840; Kim *et al. J. Org. Chem.* **1995**, *60*, 7716) utilized all glycal building blocks (Danishefsky *et al. Angew. Chem.* **1996**, *108*, 1482; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1380) for the rapid construction this complex oligosaccharide. These investigations relied on a highly convergent [3+3] coupling to generate the hexasaccharide core contained in the final target. In this approach, a flexible terminal glycal was maintained throughout the hexasaccharide construction. The glycal was then used to install the ceramide side chain present en route to globo-H glycolipid **16a** or its allyl glycoside **16b**. The synthesis of **16a** served to facilitate the proof of structure and immunocharacterization of globo-H. The allyl glycoside **16b** was employed for immunoconjugation to biocarrier proteins. The previous protocols were effective in producing *adequate* quantities of synthetic material for proof of structure, immunocharacterization, conjugation, mouse vaccinations and phase I human clinical trials. However, improved synthetic methodologies were desired to allow for efficient bioconjugation and also to provide suitable material for clinical purposes.

[0236] Difficulties associated with the allyl glycoside approach invited an alternative solution which, in general terms, is described herein, and is more specifically described for fucosyl GM1 above, and additionally for Globo-H below (Figure 7). Thus, it was conceived that a hexasaccharide could be constructed containing a glycoside that would enable linkage to carrier protein, already in place (see Figure 8). Indeed this group would already have been incorporated at the reducing end of the acceptor in the [3+3] coupling step. For successful implementation of this significant new variation of the globo-H synthesis (and other complex tumor associated antigens), it would be preferable that 1) the trisaccharide acceptor containing the glycoside construct would be readily synthesizable; 2) the glycoside construct would be compatible with the [3+3] coupling; 3) the construct, in contrast to the allyl glycoside, would survive global deprotection; and 4) efficient conjugation would be implementable.

[0237] An initial retrosynthetic analysis is shown in Figure 9. For maximum convergency, the ABC acceptor was envisioned containing the aforediscussed pentenyl

glycosidic linker. Additionally, the same DEF trisaccharide donor sector would be utilized as described previously. The hexasaccharide core would then be assembled via a convergent [3+3] ABC+DEF coupling reaction using a sulfonamido glycosidation protocol (Griffith *et al. J. Am. Chem. Soc.* **1990**, *112*, 5811; Griffith *et al. J. Am. Chem. Soc.* **1991**, *113*, 5863). Previous results had indicated that the presence of a free hydroxyl at C4 of the reducing end galactose (Figure 9, see asterisk) in the DEF donor would be necessary to direct the formation of the required β-linkage in the sulfonamido glycosidation (Park *et al. J. Am. Chem. Soc.* **1996**, *118*, 11488; Kwon *et al. J. Am. Chem. Soc.* **1998**, *120*, 1588). The sequencing of the [3+3] coupling was expected to take place as shown, owing to the higher reactivity of the equatorial C3 hydroxyl group (see bold) in the acceptor as compared to the necessary axial C4 hydroxyl group in the donor trisaccharide. Important to the strategy described herein is that, once the hexasaccharide is assembled, only protecting group manipulations would be required to reach the pro-vaccine antigen.

[0238] In general, the synthesis of the DEF trisaccharide sector is fairly concise, requiring six transformations starting from 6-*O*-TIPS galactal and tri-*O*-benzyl fluoro fucose (Park *et al. J. Am. Chem. Soc.* **1996**, *118*, 11488; Bilodeau *et al. J. Am. Chem. Soc.* **1995**, *117*, 7840; Kim *et al. J. Org. Chem.* **1995**, *60*, 7716). For purposes of a second-generation approach, the acceptor trisaccharide component can be dissected into a lactose derivative containing the desired NPG bearing a differentiated hydroxyl at C4' and an appropriate C-ring donor (Figure 9). The galactose donor monosaccharide also requires differential protection at C3, for eventual ABC+DEF coupling, and needs careful attention to efficiently allow for the required β-glycosidic linkage joining the AB+C domains.

[0239] As shown in Figure 10, the synthesis of the requisite ABC acceptor was conducted taking advantage of readily available lactose octaacetate, **17**. Conversion of **17** to the known α-bromo donor **18** (Reithal, Y. *J. Am. Chem. Soc.* **1952**, *74*, 4210; Dasgupet *et al. Carbohydr. Res.* **1994**, *264*, 155) was followed by silver carbonate mediated glycosylation with pentenyl alcohol as acceptor, to give **19** (Pent = $CH_2CH_2CH_2CH{=}CH_2$) in 75% yield on a 100 g scale (Rodriguez *et al. Aust. J. Chem.* **1990**, *43*, 665). Similar processing of **18** with silver triflate as promoter resulted in 17% yield of the desired product. Thus, with the formation of **19**,

in an early stage of the synthesis, the linker was successfully installed to be used for late stage bioconjugation.

[0240] Subsequent steps were designed to generate a free acceptor site at C4' of **19** for an eventual AB + C coupling give the ABC trisaccharide (Figure 10). Removal of the ester protecting groups in **19** to give a pentenyl lactoside was followed by a stannane mediated monobenzylation to selectively give the C3' benzyl ether (David *et al. J. Chem. Soc. Perkin Trans. I* **1981**, 1797; Maranduba *et al. Carbohydr. Res.* **1986**, *151*, 105). In a second step, the C4' and C6' hydroxyls were engaged as a benzylidene acetal to provide compound **20** as the only observable product (Jannson *et al. J. Org. Chem.* **1998**, *53*, 5629; Koeman *et al. Tetrahedron* **1993**, *49*, 5291; Qiu *et al. Liebigs Ann.* **1992**, 217). Finally, perbenzylation of the remaining hydroxyl groups in **20** and regioselective reductive cleavage of the benzylidene with sodium cyanoborohydride and anhydrous HCl gave the C4' alcohol **21** (Garegg, P.J. *Pure Appl. Chem.* **1984**, *56*, 845). Thus, starting from lactose octaacetate **17**, the AB pentenyl glycoside acceptor **21** was obtained in 7 steps and in 20% overall yield.

[0241] With large quantities of the protected pentenyl glycoside **21** available, attention was turned to the AB+C coupling to form the trisaccharide acceptor **24**. The previous synthesis of glycal **27** (Figure 10) required careful preparation of the highly activated β-fluoro donor **23** from glycal **22**. The C3 PMB ether contained in **22** was strategically incorporated to allow for eventual ABC+DEF coupling upon selective deprotection of this group. In the course of this work, it was discovered that α-**23** could be formed conveniently in high yield and on large scale. Accordingly, α-donor **23** was prepared from differentially protected glycal **22** by epoxidation, exposure to HF:pyridine to yield the *cis* fluoro-hydrin derivative and subsequent conversion of the resulting C2-hydroxyl to its benzyl ether. The anomeric α:β selectivity was demonstrated to be 10:1 and the overall yield in transforming **22** into **23** was 76%.

[0242] The effectiveness of the AB+C coupling using previously prepared β-**23** and the newly prepared α-**23** with the AB acceptor **21** was then investigated. The synthetic optimization of glycal trisaccharide **27** as a model case (see **23+26->27**) was also examined because of its presumed sensitivity to overly demanding coupling promoters. In these investigations it was discovered that the reduced reactivity of α-fluoro donors could be attenuated by conducting the couplings with highly fluorophilic promoters in judiciously chosen solvents, as summarized in

Table 1. The previous coupling procedure using the predominantly β–fluoro donor **23** and glycal **26** to give glycal trisaccharide **27** employed Muykiyama coupling conditions (Mukaiyama *et al.* *Chem. Lett.* **1981**, 431; Nicolaou *et al.* *J. Am. Chem. Soc.* **1990,** *112*, 3693; Nicolaou *et al.* *J. Chem. Soc. Chem. Commun.* **1991**, 870) and proceeded in 54% yield with modest anomeric selectivity (entry 1, Table 1). Investigations using other promoters with α-**23** are shown in entries 2 and 3, but produced little satisfaction in terms of overall efficiency. However, the preparation of glycal **27** was successfully extended to include the described α-donor **23** using strongly fluorophilic $Cp_2Zr(OTf)_2$ promotion (73% yield, entry 4). Gratifyingly, these optimized glycosidation conditions for formation of **27** were successfully applied to the AB+C coupling employing pentenyl glycoside **21** to provide trisaccharide **24** in yields that rivaled the parent reaction (80% yield, entry 6). Muykiyama coupling of β-**23** with **21** yielded 42% of trisaccharide **24** (entry 5). Satisfied with the events leading to smooth formation of large quantities of **24**, the [3+3] coupling would be investigated. The discharge of the lone PMB group in **24** could be effected in excellent yield (92%), thus completing the assembly of the desired ABC pentenyl acceptor **25**.

[0243] **Table 1.** Coupling conditions used to generate the ABC trisaccharide.

	Acceptor AB	Donor C	Promoter, Solvent	α : β selectivity	Yield, Product
1	**26**[a]	**23** (β)[a]	$SnCl_2$, $AgClO_4$, Et_2O	3:1	54% (α), 18% (β) **27**
2	**26**	**23** (10α:1β)	$Sn(OTf)_2$ Toluene:THF (5:1)	8:1	40% (α), 5% (β) **27**
3	**26**	**23** (10α:1β)	Cp_2ZrCl_2, $AgClO_4$, CH_2Cl_2:Et_2O	2.7:1	55% (α) **27**
4	**26**	**23** (10α:1β)	$Cp_2Zr(OTf)_2$, Toluene:THF (10:1)	10:1	72% (α), 8% (β) **27**
5	**21**	**23** (β)	$SnCl_2$, $AgClO_4$, Et_2O	3:1	42% (α) **24**
6	**21**	**23** (10α:1β)	$Cp_2Zr(OTf)_2$, Toluene:THF (5:1)	10:1	80% (α), 8% (β) 24

(a: see Park *et al.* *J. Am. Chem. Soc.* 1996, 118, 11488; Bilodeau *et al.* *J. Am. Chem. Soc.* **1995**, *117*, 7840; Kim *et al.* *J. Org. Chem.* **1995**, *60*, 7716).

[0244] The key step and final transformations completing the synthesis of **16c** are shown in Figure 11. Treatment of the known DEF donor **28** (see Figure 10) with MeOTf (Lönn, H. *Carbohydr. Res.* **1985,** *134*, 105; Lönn, H. *J. Carbohydr. Chem.* **1987**, *6*, 301) In the presence of

acceptor **25** smoothly provided hexasaccharide **29** in 60% yield. The configuration of the new anomeric center of **29** was confirmed to be β-configured. The [3+3] coupling yield using trisaccharide acceptor **25** was comparable to the [3+3] procedure using the glycal-based acceptor corresponding to **27**. The tremendous advantage of using **15**, however, is manifested in the steps which follow.

[0245] Global deprotection began with subjection of **29** to TBAF in order to remove the silyl ethers and the cyclic carbonate. The benzyl and sulfonamido protecting groups on the resulting penta-ol were then cleaved under the action of dissolving metal reduction. This protocol was followed by peracetylation to give the isolable hexasaccharide peracetate **30**. As in earlier steps, the pentenyl linkage proved highly reliable under the listed deprotection conditions. It is again notable by contrast that the corresponding allyl glycoside (to ultimately yield **16b**) is not stable to the reducing metal conditions required for global deprotection and therefore must be installed subsequent to deprotection. Deacetylation of **30** with methoxide yielded the fully deprotected pentenyl glycoside of globo-H, **16c**, notably poised for bioconjugation. *Importantly, in the second generation variation, progress toward **16d** from hexasaccharide construct **29** was greatly simplified because the need for additional functionalization to allow for conjugation is eliminated.*

[0246] Toward the goal of facilitating clinical evaluation of synthetic globo-H, **16c** has been conjugated to carrier protein KLH for purposes of creating a functional vaccine. The first step of this procedure involved ozonolysis of the pendant olefin, followed by reductive work-up, to give the uncharacterized aldehyde intermediate **31**, as shown in Figure 12. Reductive amination with KLH and sodium cyanoborohydride in phosphate buffer yielded vaccine glycoconjugate **16d** ($n = 3$). Covalent attachment of the carbohydrate to the proteins presumably occurs through the ε-amino groups on exposed lysine residues in KLH. Hydrolytic carbohydrate analysis of **16d** revealed approximately 350 carbohydrate residues per molecule of carrier protein.

[0247] ***2) Experimentals:***

[0248] **Peracetyl pentenyl-β-D-lactoside (19).** To a cooled (ice bath) suspension of lactose octaacetate (100.0 g, 147.7 mmol), glacial acetic acid (30 mL) and acetic anhydride (30 mL) was added 100 mL of 30% HBr in AcOH dropwise over a period of 60 minutes. The

reaction mixture stirred for 1 hour and the ice bath was removed. Upon stirring for an additional 2 hours at room temperature, the mixture became a homogeneous yellow solution. The solution was diluted with H_2O (1000 mL) and extracted with $CHCl_3$ (3x400 mL). The organic extracts were washed with H_2O (2x1000 mL), satd. $NaHCO_3$ (3x500 mL), dried over $MgSO_4$ and concentrated. The product was azeotroped with anhydrous benzene and dried under high vacuum to yield 98.8 g (96%) of the lactosyl bromide which was used without further purification.

[0249] To a suspension of Ag_2CO_3 (100 g, 362.6 mmol), freshly activated molecular sieves (15 g) and a crystal of I_2 in 400 mL CH_2Cl_2 was added pentenyl alcohol (5.0 equiv., 73.4 mL) and then the lactosyl bromide (98.8 g, 141.4 mmol) in 400 mL of CH_2Cl_2. After stirring in the dark at room temperature for 16 hours, the reaction was filtered through a plug of Celite with additional CH_2Cl_2 and concentrated to a yellow oil which was purified by flash column chromatography (10% EtOAc/hexanes→50% EtOAc/hexanes) to yield 74.7 g (75%) of the pentenyl lactoside as a white foam. $[\alpha]_{22}^{D}$ -48.9° (*c* 7.5, $CHCl_3$); IR (film $CHCl_3$) 2941, 1751, 1369, 1224, 1054 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.60 (m, 1H), 5.17 (d, 1H, J=2.7 Hz), 5.02 (m, 1H), 4.93 (dd, 1H, J=7.9, 10.3 Hz), 4.85 (d, 1H, J=1.6 Hz), 4.78 (m, 2H), 4.71 (dd, 1H, J=9.6, 7.9 Hz), 4.30 (m, 3H), 3.93 (m, 3H), 3.66 (m, 3H), 3.45 (m, 1H), 3.30 (m, 1H), 1.98 (s, 3H), 1.94 (s, 3H), 1.91 (m, 2H), 1.89 (s, 3H), 1.88 (s, 6H, 2xCH3), 1.87 (s, 3H), 1.79 (s, 3H), 1.49 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.33, 170.28, 170.09, 170.00, 169.74, 69.54, 169.01, 137.72, 115.00, 101.01, 100.51, 76.27, 72.76, 72.48, 71.64, 70.94, 70.58, 69.23, 69.01, 66.52, 61.97, 60.73, 29.75, 28.49, 20.80, 20.75, 20.64, 20.57, 20.45. FAB-HRMS calc'd for $C_{31}H_{44}O_{18}Na^+$; 727.2425. Found; 727.2418.

[0250] Pent-4-enyl 3'-*O*-benzyl-4', 6'-*O*-benzylidenyl-β-D-lactoside (20). Peracetylated pentenyl lactoside, **8**, (18.2 g, 25.8 mmol) was dissolved in 300 mL of anhydrous MeOH and 2.0 mL of NaOMe (25% in MeOH) was added. The reaction stirred at rt for 16 hours and was neutralized with Dowex-H^+ (pH 5-6). The reaction was filtered with additional MeOH and concentrated to a white solid, **19a**, (10.6 g, quantitative) which was used without further purification: ^1H NMR (D_2O, 400 MHz) δ 5.81 (m, 1H), 5.00 (dd, 1H, J=17.3, 1.9 Hz), 4.92 (dd, 1H, J=8.9 Hz), 4.74 (m, 1H), 4.39 (d, 1H, J=8.0 Hz), 4.35 (d, 1H, J=7.8 Hz), 3.72-3.42 (m, 12H),

3.21 (m, 1H0, 2.06 (m, 2H), 1.63 (m, 2H); ^{13}C NMR (D_2O, 100 MHz) δ 141.27, 117.31, 105.42, 104.54, 80.85, 77.84, 77.24, 76.92, 75.33, 75.00, 73.44, 72.47, 71.03, 63.52, 62.56, 31.83, 30.48. The hepta-ol **19a** (1.14 g, 2.8 mmol) and dibutyltin oxide (0.76 g, 3.1 mmol) were heated at reflux in benzene (70 mL) with azeotropic water removal for 15 h. The mixture was doubled in concentration, cooled to room temperature, and benzyl bromide (0.69 ml, 5.8 mmol) and Bu_4NI (1.03 g, 2.8 mmol) were added. The mixture was heated at reflux 3.5 h, cooled, silica gel was added to the flask, and the solvent was evaporated. The residue was applied to a column of silica gel, tin by-products were removed by flushing with hexanes, and elution (5 % MeOH in CH_2Cl_2) gave the pure 3'-O-benzyl ether (0.76 g, 54 %) as a white foam: $[\alpha]_{22}^{D}$ +36.7° (*c* 2.73, $CHCl_3$); IR (film $CHCl_3$) 3371, 2924, 2880, 1372, 1157, 1074 cm^{-1}; ^1H NMR (MeOH-d_4, 400 MHz) δ 7.46-7.40 (m, 2H), 7.35-7.20 (m, 3H), 5.92-5.72 (m, 1H), 5.08-4.93 (m, 2H), 4.76 (d, 1H, J=11.8 Hz), 4.65 (d, 1H, J=11.8 Hz), 4.38 (d, 1H, J=7.8 Hz), 4.28 (d, 1H, J=7.8 Hz), 4.02 (d, 1H, J=2.9 Hz), 3.95-3.63 (m, 6H), 3.61-3.48 (m, 4H), 3.43-3.20 (m, 3H), 2.20-2.10 (m, 2H), 1.78-1.65 (m, 2H); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 139.77, 139.47, 129.29, 129.08, 128.64, 115.19, 105.02, 104.23, 82.17, 80.74, 76.88, 76.40, 76.35, 74.71, 72.55, 71.81, 70.23, 67.02, 62.44, 61.91, 31.22, 30.07. FAB-HRMS calc'd for $C_{24}H_{36}O_{11}Na^+$; 523.2155. Found; 523.2137.

[0251] The 3'-O-benzyl ether (0.6 g, 1.20 mmol) was dissolved in acetonitrile and DMF (5:2, 7 mL), and benzaldehyde dimethylacetal (0.47 mL, 3.1 mmol) and CSA (14 mg, 60 μmol) were added. After stirring 16 h at room temperature, the mixture was diluted with CH_2Cl_2 and washed with sat. $NaHCO_3$. The organic extracts were dried ($MgSO_4$), evaporated, and following addition of ether (100 mL) to the resulting residue, the pure **20** was recovered by filtration (0.51 g, 72 %): $[\alpha]_{22}^{D}$ +111° (*c* 2.21, MeOH); IR ($CHCl_3$ film) 3440, 2872, 1368, 1163, 1109, 1048, 1005 cm^{-1}; ^1H NMR (MeOH-d_4, 400 MHz) δ 7.55-7.11 (m, 10H), 5.82-5.69 (m, 1H), 5.45 (s, 1H), 4.98-4.83 (m, 2H), 4.64 (d, 2H, J=3.0 Hz), 4.40 (d, 1H, J=7.9 Hz), 4.23 (d, 1H, J=3.4 Hz), 4.18 (d, 1H, J=7.8 Hz), 4.15-3.98 (m, 2H), 3.87-3.66 (m, 4H), 3.55-3.10 (m, 7H), 2.20-2.10 (m, 2H), 1.65-1.53 (m, 2H); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 139.76, 139.49, 139.47, 129.86, 129.30, 129.07, 129.03, 128.72, 127.35, 115.19, 104.69, 104.28, 102.03, 80.63, 80.17, 76.37, 76.28, 74.77, 74.73, 72.84, 70.86, 70.25, 68.17, 61.70, 31.22, 30.07. FAB-HRMS calc'd for $C_{31}H_{40}O_{11}Na^+$; 611.2468. Found; 611.2465.

[0252] **Pent-4-enyl 2, 2', 3, 3', 6, 6'-hexa-*O*-benzyl-β-D-lactoside (21).** The tetraol **20** (0.51 g, 0.87 mmol) and Et_4NI (0.12 g, 0.43 mmol) were dried (azeotropic distillation with benzene), dissolved in DMF (5 mL) and cooled to 0°C. Benzyl bromide (0.83 mL, 7.0 mmol) was added followed by NaH (0.22 g, 60 %, 5.6 mmol) and the mixture was allowed to warm to room temperature over 14 h. The mixture was diluted with ethyl acetate, washed with water, the organic layer was dried ($MgSO_4$) and evaporated. Purifiction of the residue by chromatography on silica gel (4:1→2:1 hexanes:ethyl acetate) gave pure pentabenzyl lactoside as a white foam (0.80 g, 97 %): $[\alpha]_{22}^{D}$ +129° (*c* 1.63, $CHCl_3$); IR ($CHCl_3$ film) 3030, 2866, 1453, 1365, 1096, 1063, 1028, 911 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.50-7.05 (m, 30H), 5.80-5.65 (m, 1H), 5.38 (s, 1H), 5.10 (d, 1H, J=10.6 Hz), 4.99-4.60 (m, 9H), 4.47 (d, 1H, J=12.1 Hz), 4.38 (d, 1H, J=7.8 Hz), 4.30 (d, 1H, J=7.8 Hz), 4.25 (d, 1H, J=12.1 Hz), 4.12 (d, 1H, J=13 Hz), 3.94 (d, 1H, J=3.4 Hz), 3.92-3.60 (m, 6H), 3.54 (dd, 1H, J=8.8 Hz, 9.2 Hz), 3.46 (dd, 1H, J=2.6 Hz, 7.0 Hz), 3.40-3.23 (m, 3H), 2.85 (s, 1H), 2.22-2.00 (m, 2H), 1.75-1.60 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.92, 138.63, 138.51, 138.04, 128.80, 128.52, 128.31, 128.24, 128.17, 128.13, 128.06, 128.03, 128.00, 127.69, 127.65, 127.54, 127.49, 127.38, 127.30, 126.52, 114.84, 103.59, 102.83, 101.30, 83.01, 81.81, 79.60, 78.76, 77.65, 75.73, 75.22, 75.05, 74.97, 73.61, 72.91, 71.56, 69.27, 68.90, 68.27, 66.28, 30.18, 28.89. FAB-HRMS calc'd for $C_{59}H_{64}O_{11}Na^+$; 971.4346. Found; 971.4375.

[0253] The benzylidene (0.63 g, 0.66 mmol) was dissolved in THF (6.6 mL) and stirred with freshly activated 4ÅMS (0.25 g) 10 min at room temperature. In one portion $NaCNBH_3$ (0.21 g, 3.3 mmol) was added followed by anhydrous HCl (2.0 M Et_2O), dropwise until the mixture no longer bubbled (approx. 2 mL). After stirring and additional 10 min, the mixture was passed through a plug of Celite washing with ethyl acetate, the filtrate was washed with sat. $NaHCO_3$ and brine, dried ($MgSO_4$) and the organic layers evaporated. Purification by column chromatography (9:1 hexanes:ethyl acetate) gave pure **21** as white solid (0.49 g, 79 %): $[\alpha]_{22}^{D}$ +200° (*c* 1.05, $CHCl_3$); IR ($CHCl_3$ film) 3474, 3062, 3029, 2869, 1453, 1364, 1094, 1028 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.40-7.06 (m, 30H), 5.80-5.66 (m, 1H), 5.02-4.85 (m, 3H), 4.81 (d, 1H, J=11.0 Hz), 4.75-4.54 (m, 6H), 4.67 (d, 1H, J=12.2 Hz), 4.42-4.26 (m, 5H), 3.94 (s, 1H), 3.92-3.81 (m, 2H), 3.71 (dd, 1H, J=10.7 Hz, 4.1 Hz), 3.64 (d, 1H, J=10.6 Hz), 3.57 (dd, 1H, J=9.4 Hz, 5.5 Hz), 3.55-3.42 (m, 3H), 3.38 (dd, 1H, J=5.2 Hz, 9.6 Hz), 3.36-3.21 (m, 4H), 2.32

(s, 1H), 2.15-2.02 (m, 2H), 1.74-1.60 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.04, 138.54, 138.52, 138.23, 138.09, 137.96, 137.81, 128.33, 128.15, 127.93, 127.66, 127.50, 114.80, 103.50, 102.43, 82.79, 81.68, 80.99, 79.27, 76.52, 75.22, 75.10, 74.99, 74.83, 73.37, 72.99, 72.67, 71.86, 69.10, 68.32, 68.16, 66.00, 30.11, 28.83. FAB-HRMS calc'd for $C_{59}H_{66}O_{11}Na^+$; 973.4503. Found; 973.4513.

[0254] α-**Fluoro donor (23).** A solution of 3-*O*-PMB-4,6-Di-*O*-benzyl-galactal (2.24 g, 5.02 mmol) in dry CH_2Cl_2 (5 ml) under N_2 at 0°C was treated with dimethyldioxirane (0.11 M, 47 ml), and the mixture was stirred until all of the glycal was consumed (~ 1h, TLC 30% EtOAc in hexane) Note: *Elevated temperature and/or excess of DMDO will prompt oxidation of the PMB group and lower reaction yield.* The solvents were evaporated under vacuum at 0°C and the residue was kept under high vacuum. The flask containing galactal epoxide was charged with freshly prepared 4 A molecular sieves (2 g), dry THF (50 ml) and cooled to 0°C. HF/Pyr complex (0.79 ml, ~5 equiv.) was added dropwise via syringe. The reaction mixture was left overnight to slowly reach room temperature and quenched with Et_3N (1.27 g, ~ 2.5 equiv.) to reach pH ~ 7. The mixture was filtered through a pad of anhydrous $MgSO_4$ and rinsed three times with 50 ml of EtOAc. The filtrate was washed with water (50 ml) and saturated $NaHCO_3$ solution (50 ml), dried over $MgS0_4$ and concentrated to dryness. Flash column chromatography (EtOAc/hexanes, 2/1) gave 2.06 g (85% yield) of fluorohydrin as a mixture of anomers α:β = 10 : 1. ^{19}F NMR ($CDCl_3$, 376 MHz, C_6F_6 as external standard) δ 9.7 (dd, α, J = 54.4, 25.0 Hz) 20.0 (dd, β, J = 53.9, 13.1 Hz); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.38 – 7.24 (m, 12H), 6.90 (d, 2H, J = 8.7 Hz), 5.70 (dd, 1H, J = 54.4, 2.8 Hz), 4.89 and 4.57 (two d, 2H, J = 11.3 Hz), 4.70 and 4.54 (AB q, 2H, J = 11.2 Hz), 4.54 and 4.46 (AB q, 2H, J = 11.8 Hz), 4.17 (AMX octet, 1H, J = 2.8, 10.1, 25.0 Hz), 4.13 (br t, 1H, J = 6.8 Hz), 4.06 (d, 1H, J = 1.5 Hz), 3.81 (s, 3H), 3.74 (dd, 1H, J = 2.6, 10.1 Hz), 3.60 (m, 2H).

[0255] The above mixture (8.29 g, 17.2 mmol) was dissolved in dry DMF (100 ml) containing

freshly prepared 4 A molecular sieves (3 g) under N_2 at 0°C, treated with benzyl bromide (4.41 g, 25.8 mmol, 1.5 equiv.) and finally with NaH (1.24 g, 60% dispersion in oil, 30.86 mmol, 1.8 equiv.), and stirred overnight at room temperature. The reaction was quenched with glacial acetic acid (0.93 g, 0.9 equiv.) and the mixture filtered through a pad of anhydrous $MgSO_4$ with

EtOAc (4x50 ml). The organic solution was washed with water (4 x 50 ml), dried ($MgSO_4$) and concentrated *in vacuo*. Flash column chromatography of the residue (hexane/EtOAc, 4/1) gave 9.36 g (95%) of the title compound as colorless liquid with the same ratio of anomers α :β = 10 : 1 as the starting fluorohydrin. ^{19}F NMR ($CDCl_3$, 376 MHz, C_6F_6 as external standard) δ 11.5 (dd, α, J = 53.7, 25.2 Hz), 22.8 (dd, β, J = 53.4, 13.0 Hz). For analytical purpose 50 mg of pure α anomer was obtained using preparative HPLC. $[\alpha]_{22}^{D}$ - 54.5° (*c* 0.55, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz), δ 7.38–7.24 (m, 17H), 6.88 (d, 2H, J=8.6 Hz), 5.58 (dd, 1H, J=53.7, 2.7 Hz), 4.93 (d, 2H, J=11.34 Hz), 4.56 (d, 2H, J=11.34 Hz), 4.85 (AB q, 2H, J = 11.78 Hz), 4.72 (AB q, 2H, J = 11.78 Hz), 4.73 (AB q, 2H, J = 11.3 Hz), 4.68 (AB q, 2H, J = 11.3 Hz), 4.47 (AB q, 2H, J = 11.84 Hz), 4.41 (AB q, 2H, J = 11.84 Hz), 4.09 (br t, 1H, J = 6.5 Hz), 4.02 (AMX m, 1H, J = 2.7, 10.05, 25.2 Hz), 3.98 (app s, 1H), 3.92 (dd, 1H, J = 2.64, 10.05 Hz), 3.81 (s, 3H), 3.54 and 3.52 (ABX m, 2H, J=9.3, 6.05, 7.0 Hz); ^{13}C NMR ($CDCl_3$, 125MHz) δ 159.20, 138.35, 138.08, 137.71, 130.43, 129.18, 128.39, 128.25, 128.14, 127.92, 127.8, 127.78 , 127.66, 113.81, 106.25 (d, J = 229.0 Hz), 78.09, 75.65 (d, J = 23.5 Hz), 74.79 ($ArCH_2$), 74.29, 73.70 ($ArCH_2$), 73.45 ($ArCH_2$), 72.71 ($ArCH_2$), 71.70 (d, J = 2.7 Hz) 68.26, 55.24 (CH_3O); MS (NH_3) 586 ($[M + NH_4]^+$).

[0256] **PMB trisaccharide (24).** A mixture of lactoside (**21**) (402 mg, 0.423 mmol) and fluoro donor (**23**) (485 mg, 0.846 mmol, 2 equiv.) was azeotroped with anhydrous benzene (3x10 ml) and further dried on high vacuum for 3 h. The above mixture was dissolved in toluene (3.8 ml) and transferred via cannula to a flask containing freshly prepared 4 Å molecular sieves (0.68 g) under N_2, treated with 2,6-di-tert-butylpyridine (143 μl) and cooled to -20 °C. $(Cp)_2Zr(OTf)_2$ (225 mg, 0.381 mmol, 0.9 equiv.) was suspended in THF (0.38 ml) and added via a cannula to the reaction mixture. The reaction was stirred for 72 h at 7 °C in darkness. The reaction mixture was diluted with EtOAc (10 ml) and filtered through a pad of anhydrous $MgSO_4$ with EtOAc (3x 10mL). The filtrate was washed with satd. $NaHCO_3$ solution (2 x 10 ml), dried over $MgSO_4$, and concentrated to dryness. Flash column chromatography (2% Et_2O/CH_2Cl_2) gave 509 mg (80%) of desired α-product (**24**) and 51 mg (8%) of β-product. $[\alpha]_{22}^{D}$ +24.6° (*c* 3.90, $CHCl_3$); IR ($CHCl_3$ film) 3062, 3029, 2919, 2868, 1612, 1513, 1496, 1364, 1303, 1248, 1028 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40-6.95 (m, 49 H), 6.69 (d, 1H, J=8.5 Hz), 5.73 (m, 1H), 5.00-4.93 (m, 2H), 4.92-4.84 (m, 2H), 4.82-4.73 (m, 2H), 4.72-4.63 (m, 5H), 4.61 (d, 1H, J=13.0 Hz), 4.48-

4.35 (m, 5H), 4.34-4.24 (m, 4H), 4.16 (d, 2H, J=6.8 Hz), 4.07 (dd, 1H, J=8.8 Hz), 4.02-3.80 (m, 8H), 3.78-3.60 (m, 3H), 3.68 (s, 3H), 3.60-3.35 (m, 6H), 3.35-3.18 (m, 4H), 3.12-3.04 (m, 1H), 2.06 (m, 2H), 1.65 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.76, 139.66, 139.45, 139.26, 139.16, 139.09, 138.92, 138.57, 138.52, 131.39, 129.30, 128.95, 128.70, 128.60, 129.30, 128.08, 127.95, 115.35, 114.02, 104.05, 103.35, 101.25, 83.14, 82.17, 79.91, 79.71, 77.77, 77.04, 75.69, 75.58, 75.46, 75.33, 74.17, 73.75, 73.54, 73.48, 72.65, 72.54, 69.91, 69.71, 68.80, 68.33, 68.19, 55.11, 30.14, 28.86; FAB-HRMS calc'd for $C_{94}H_{102}O_{17}Na^+$; 1525.7014. Found; 1525.6996.

[0257] **Trisaccharide acceptor (25).** A solution of PMB trisaccharide (**24**) (445 mg, 0.296 mmol) in methylene chloride (10 ml) at 0 °C was treated with phosphate buffer (1.5 ml, pH = 7.4) and DDQ (89 mg, 1.3 equiv.) and stirred at 0 °C for 5 h. The reaction mixture was diluted with EtOAc (50 ml), washed with satd. $NaHCO_3$ solution (2 x 20 ml) and water (20 ml), dried over $MgSO_4$, and concentrated to dryness. The crude material was purified by flash column chromatography (4% ether in methylene chloride) to give 344 mg (84%) of deprotected trisaccharide (**25**) as a colorless oil. $[\alpha]_{22}^{D}$ +28.2° (*c* 5.70, $CHCl_3$); IR ($CHCl_3$ film) 3570, 3062, 3029, 2913, 2868, 1496, 1453, 1364, 1208, 1095 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.77-7.06 (m, 45H), 5.73 (m, 1H), 5.01 (dd, 1H, J=5.5, 3.3 Hz), 4.95 (dd, 1H, J=5.8, 2.6 Hz), 4.90 (m, 1H), 4.78 (d, 1H, J=10.9 Hz), 4.75 (d, 1H, J=11.4 Hz), 4.70-4.59 (6d, 6H), 4.47-4.37 (m, 5H), 4.28 (m, 3H), 4.19 (s, 2H), 4.08-3.91 (m, 6H), 3.85 (m, 2H), 3.69 (m, 5H), 3.66 (1H, d, J=11.0 Hz), 3.50-3.19 (m, 9H), 3.10 (dd, 1H), 2.07 (m, 2H), 1.79 (d, 1H, OH), 1.65 (d, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.36, 138.72, 138.63, 138.52, 138.41, 138.29, 138.19, 138.07, 137.98, 128.35, 128.20, 128.06, 127.97, 127.66, 127.54, 127.08, 114.82, 103.55, 102.67, 99.58, 82.93, 81.67, 81.55, 79.32, 77.61, 76.90, 75.13, 75.02, 74.96, 74.80, 73.08, 72.99, 72.91, 72.01, 69.95, 69.22, 69.15, 68.34, 67.73, 67.57, 60.35, 30.19, 28.92; FAB-HRMS calc'd for $C_{86}H_{94}O_{16}Na^+$; 1405.6439. Found; 1405.6385.

[0258] **Hexasaccharide (29).** The thioethyl donor **28** (543 mg, 0.425 mmol) and acceptor **25** (587 mg, 0.425 mmol) were combined, azeotroped with anhydrous benzene (5x5 mL) and placed under high vacuum for 5 hours. The mixture was then dissolved in 3.5 mL CH_2Cl_2 and 7.0 mL Et_2O, treated with freshly prepared molecular sieves and cooled to 0 °C. Methyl triflate (3.0 equiv., 144 μL) was added in one portion and the reaction stirred at 0 °C for 3 hours. Another 144 μL of MeOTf was added and the reaction was allowed to stir for an additional 2

hours at 5 °C. The reaction was quenched by the addition of solid $NaHCO_3$, filtered through Celite with EtOAc, concentrated and purified by HPLC (17% EtOAc/hexanes) to give 663 mg (60%) of hexasaccharide as a white foam. $[\alpha]_{22}^{D}$ –9.7° (*c* 1.00, $CHCl_3$); IR ($CHCl_3$ film) 3533, 3343, 3087, 3030, 2940, 2865, 1790, 1496, 1453, 133, 1095 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.76 (d, 2H, J=7.5 Hz), 7.45-7.00 (m, 63H), 5.84 (m, 1H), 5.20 (s, 1H), 5.11 (d, 1H, J=3.2 Hz), 5.09 (d, 1H, J=3.6 Hz), 5.05 (d, 1H, J=3.3 Hz), 5.03 (m, 1H), 4.92 (m, 2H), 4.86 (d, 1H, J=6.0 Hz), 4.82 (m, 2H), 4.78 (1H, d, J=2.2 Hz), 4.74-4.61 (m, 8H), 4.53-4.44 (4d, 4H), 4.38-4.30 (m, 4H), 4.18-3.82 (m, 20H), 3.76-3.66 (m, 5H), 3.66-3.60 (m, 2H), 3.58-3.52 (m, 2H), 3.48-3.40 (m, 2H), 3.38-3.32 (m, 2H), 3.29-3.25 (m, 3H), 3.06 (dd, 1H, J=10.2 Hz), 2.86 (s, 1H), 2.74 (m, 1H), 2.16 (m, 2H), 1.74 (m, 2H), 1.23 (s, 3H, J=6.5 Hz), 1.16-1.07 (m, 42H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.49, 140.71, 139.37, 138.96, 138.72, 137.70, 138.66, 138.55, 138.42, 138.37, 138.10, 138.07, 138.04, 137.88, 132.07, 128.89, 128.64, 128.50, 128.27, 128.16, 128.04, 127.86, 127.68, 127.53, 127.34, 127.20, 114.79, 103.49, 103.14, 102.61, 99.63, 99.12, 97.79, 82.26, 81.61, 81.34, 80.45, 79.36, 78.95, 78.26, 77.82, 77.64, 77.45, 77.24, 77.16, 76.83, 76.45, 75.39, 75.28, 75.12, 74.98, 74.89, 74.78, 73.94, 73.13, 72.94, 72.92, 72.52, 71.91, 71.81, 71.25, 71.11, 69.35, 69.23, 69.18, 68.18, 68.11, 68.01, 67.77, 67.54, 61.98, 61.72, 56.03, 30.16, 28.88, 18.01, 18.00, 17.95, 17.92, 11.85, 11.82; LRMS (FAB) calc'd for $C_{150}H_{185}NO_{32}SSi_2Na^+$ 2624.

[0259] **Peracetate of globo-H pentenyl glycoside (30).** To a solution of the hexasaccharide (585 mg, 0.224 mmol) in THF (10 mL) was added TBAF (1.0 M THF, 10 equiv., 2.24 mL). The reaction stirred at rt for 3 days, poured into ice water and extracted with EtOAc (3x50 mL). The organic extracts were washed with satd. $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated to an oil which was purified through a short plug of silica gel with EtOAc. The resulting triol was dissolved in anhydrous MeOH (8 mL) and sodium methoxide was added (0.25 mL of a 25% solution in MeOH). The reaction stirred at rt for 18 hours, neutralized with Dowex-H^+, filtered with MeOH washings and concentrated. To the resulting white solid was added THF (2.0 mL) and condensed liquid NH_3 (~25 mL) at -78 °C. Sodium (~500 mg) was added and the resulting blue solution stirred at -78 °C for 2 hours. The reaction was quenched with anhydrous MeOH (~10 mL), brought to rt and concentrated under a stream of dry N_2 to a volume of ~5 mL. The reaction was neutralized with Dowex-H^+, filtered with MeOH washing and concentrated to a white solid. The white solid was dissolved in 5.0 mL

pyridine and 5.0 mL CH_2Cl_2 and cooled to 0 °C. A crystal of DMAP was added followed by acetic anhydride (5.0 mL). The ice bath was removed and the reaction stirred at rt overnight. Concentration followed by purification by flash column chromatography (gradient elution 75% EtOAc/hexanes→100% EtOAc→5% MeOH/EtOAc) gave 168 mg (42%) of **30** as a white solid: $[\alpha]_{22}^{D}$ 4.37° (*c* 1.85, $CHCl_3$); IR ($CHCl_3$ film) 2939, 1747, 1370, 1229, 1066 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.66 (d, 1H, J=6.5 Hz), 5.77 (m, 1H), 5.58 (d, 1H, J=3.2 Hz), 5.47 (d, 1H, J=3.5 Hz), 5.39 (d, 1H, J=3.2 Hz), 5.29 (dd, 1H, J=10.9, 3.0 Hz), 5.24-5.06 (m, 5H), 5.04-5.02 (m, 1H), 4.99-4.85 (m, 4H), 4.74 (dd, 1H, J=10.9, 2.9 Hz), 4.53-4.40 (m, 5H), 4.36 (m, 1H), 4.26 (dd, 1H, J=10.6, 3.4 Hz), 4.18-4.03 (m, 6H), 3.99-3.96 (m, 2H), 3.87-3.81 (m, 3H), 3.77-3.74 (m, 1H), 3.51-3.45 (m, 1H), 3.03 (m, 1H), 2.16 (s, 3H), 2.15 (s, 3x3H), 2.13-2.11 (m, 2H), 2.10 (s, 3H), 2.09 (s, 3H), 2.08 (s, 2x3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.04 (s, 2x3H), 2.00 (s, 3H), 1.97 (s, 2x3H), 1.89 (s, 3H), 1.65 (m, 2H), 1.62 (s, 3H), 1.14 (d, 3H, J=6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.31, 171.55, 170.78, 170.61, 170.57, 170.48, 170.41, 170.30, 170.08, 169.75, 169.61, 169.57, 169.44, 168.96, 137.76, 115.07, 102.05, 101.29, 100.45, 99.23, 98.74, 94.29, 77.24, 77.16, 76.07, 73.68, 73.40, 73.17, 72.63, 72.34, 71.85, 71.77, 71.56, 71.34, 70.83, 70.71, 70.19, 70.08, 69.32, 69.03, 68.88, 68.09, 68.01, 67.59, 67.32, 64.48, 29.80, 28.54, 23.12, 20.90, 20.88, 20.82, 20.74, 20.73, 20.72, 20.71, 20.64, 20.62, 20.55, 20.54, 20.49, 15.91; FAB-HRMS calc'd for $C_{77}H_{107}NO_{47}Na^+$;1820.5911. Found; 1820.5994.

[0260] **Globo-H, pentenyl glycoside (16c).** The peracetate (20 mg, 0.011 mmol) was dissolved in anhydrous MeOH (2.0 mL) and 100 μL of sodium methoxide was added (25% solution in MeOH). The reaction stirred at rt for 18 hours, was neutralized with Dowex-H^+ (~pH 6-7), filtered with MeOH washings, concentrated and purificated using RP silica gel (H_2O →1% MeOH/H_2O) then P-2 Gel (H_2O elutent) to yield 12 mg (99%) of a white solid. $[\alpha]_{22}^{D}$ 3.00° (*c* 1.00, MeOH); IR 3374, 2930, 1641, 1372, 1070 cm^{-1}; ^1H NMR (MeOH, 400 MHz) δ 5.79 (m, 1H), 5.18 (d, 1H, J=3.9 Hz), 4.98 (dm, 1H, J=7.2 Hz), 4.91 (m, 1H), 4.87 (m, 1H), 4.51 (s, 1H), 4.49 (d, 1H, J=1.4 Hz), 4.41-4.36 (m, 2H), 4.24-4.20 (m, 4H), 4.10 (d, 1H, J=2.5 Hz), 4.06-4.00 (m, 3H), 3.94 (s, 1H), 3.87-3.45 (m, 22H), 3.35-3.31 (m, 2H), 3.19 (t, 1H, J=8.8 Hz), 2.10 (m, 2H), 1.96 (s, 3H), 1.66 (m, 2H), 1.19 (d, 3H, J=6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.53, 139.53, 115.27, 105.50, 105.44, 104.30, 103.96, 102.81, 101.07, 81.29, 80.59, 80.04, 79.16, 78.00, 76.81, 76.57, 76.49, 76.45, 76.39, 75.57, 74.89, 74.69, 73.58, 72.64, 72.49,

71.56, 70.65, 70.63, 70.38, 70.31, 69.70, 68.13, 62.63, 62.59, 61.94, 61.62, 53.11, 49.90, 31.29, 30.14, 23.55, 16.76. FAB-HRMS calc'd for $C_{43}H_{73}NO_{30}Na^{+}$; 1106.4115. Found; 1106.4105.

[0261] *C. Example 3: Preparation of glycoamino acids and inventive glycopeptides – n-Pentenyl glycoamino acids:*

[0262] **1) Discussion of Synthetic Methods:**

[0263] a. Horner-Emmons olefination with protected glycine derived phosphonate

[0264] In general, it was desired to incorporate the two antigens described above, fucosyl GM1 and globo-H into glycopeptides. In one embodiment, as shown in Figure 13, a tranformation utilizing catalytic asymmetric hydrogenation of glycosylated amide esters may be used. The new approach anticipated a Horner-Emmons olefination of the protected aldehyde with a suitably protected glycine derived phosphonate to give an enamide ester. Subsequent catalytic asymmetric hydrogenation would hopefully yield diastereromerically pure glycoamino acid.

[0265] In but one example, an inventive glycoamino acid, based on a peracetylated lactose derivatives was prepared. Specifically the required lactose derived enamide ester substrate was prepared. The required lactose derived enamide ester substrate was prepared according to Figure 14. Ozonolysis of the NPG **32** (Allen *et al., Chem. Eur. J.* **2000,** *6*, 1366) followed by reductive work-up gave the corresponding aldehyde derivative. The crude aldehyde was then subjected to Horner-Emmons olefination using tetramethylguanidine and phosphonate **33**. Phosphonate **33**, with *N*-Boc and 2-(trimethylsilyl)ethyl ester (TSE) protection (Schmidt *et al., Synthesis* **1984,** *53*; Kawai *et al., Chem. Lett.* **1990**, 577) was chosen because of the need for the resulting glycoamino acids to be orthogonally suitable for peptide couplings in the presence of acetate carbohydrate protecting groups. The enamide ester **34** was obtained as a single geometric isomer in 88% yield for the 2-step procedure.

[0266] In one preferred embodiment, conditions for asymmetric hydrogenation of enamide ester **34** are detailed. The (*S, S*) ligand isomer of ethyl DuPHOS catalyst precursor was utilized, which has been well characterized in these types of systems to give the (*S*)-isomer in the amino acid product. The protected glycoamino acid was obtained in 98% yield and was determined to have been formed with a diastereomeric ratio (dr) of >20:1. Remarkably, the *t*-Boc protons are nearly baseline resolved and, in the asymmetric reaction, the minor isomer could

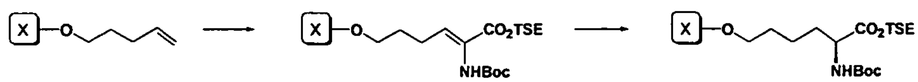
not be detected. ^{13}C analysis also supports the conclusion that the minor isomer is not formed within the limit of NMR detection. Hydrogenation of **34** with an achiral catalyst (Pd/C, MeOH) produced a 1:1 mixture of R and S configured **35**, providing a comparison for diastereomeric ratio determination. This reaction also indicates that chirality transfer to yield **35** occurs from the chiral ligand and not carbohydrate derived substrate control. A final step to be performed prior to moving to synthesis and assembly of tumor antigens was that of demonstrating deprotectability of the blocking groups contained in the amino acid side chain. In the event, reaction of **35** with TBAF in THF gave acid **36**, suitably prepared for peptide coupling, in 93% yield.

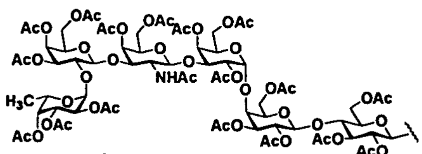
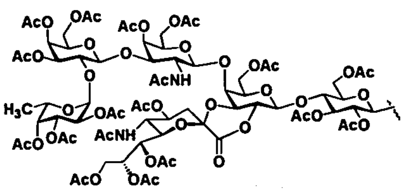
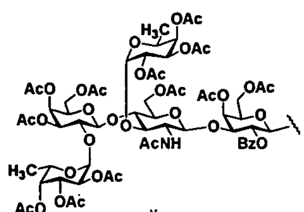
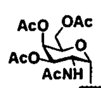
[0267] With the general methodology demonstrated in the lactose model, in other preferred embodiments, advanced hexasaccharides **37** and **38**, as well as other antigens of interest, were investigated. As shown in Table 2, olefination of the peracetylated *n*-pentenyl glycoside of Globo-H, **37**, under the same conditions as those used in Figure 14, provided the corresponding enamide ester **41** in 72% yield as a single isomer and provided fucosyl GM1 hexasaccharide 16 in 10-22% yield. Notably, through use of the (*S*, *S*)-Et-DuPHOS-Rh^+ catalyst system, the hydrogenation of **41** and **42** proceeded in excellent yield producing **45** and **46** as single diastereomers by ^1H NMR analysis. Compounds **45** and **46** represent the first examples of synthetic glycoamino acids containing the complex oligosaccharides Globo-H and fucosyl GM_1.

[0268] Similar transformations on two other clinically promising antigens to generate their corresponding glycoamino acids were also performed in certain other embodiments. As discussed previously, $Lewis^y$ (Le^y) oligosaccharide has been identified as an important antigen for eliciting antibodies against colon, liver, prostate and ovarian carcinomas (Lloyd *et al.*, *Am. J. Clin. Path.* **1987**, *87*, 129; Lloyd *et al.*, *Cancer Biol.* **1991**, *2*, 421; Yin *et al.*, *Int. J. Cancer*, **1996**, *65*, 406). Previously, both a Le^y -KLH conjugate vaccine (Danishefsky *et al. J. Am. Chem. Soc.* **1995**, *117*, 5701) and a clustered Le^y glycopeptide (of natural α-*O*-linked configuration) glycoconjugate attached to either a glycolipid or KLH have been prepared, and have initiated human clinical trials against ovarian cancer with these vaccines have been initiated (Kudryashov *et al.*, *Cancer Immunol. Immunother.* **1998**, *45*, 281; Sabbatini *et al.*, *Int. J. Cancer* **2000**, *87*, 79).

[0269] The results starting with both Le^y *n*-pentenyl glycoside **39** and the α-linked *n*-pentenyl glycoside of the Tn antigen **40** (GalNAc) are presented in Table 2. The pentasaccharide **39** was available as an *intermediate in the synthesis of* Le^y *glycopeptide cluster* and consequently illustrates the potential advantage of this strategy. Thus, if immunogenicity is retained in the artificial constructs, these NPG derived glycoamino acids offer a much shorter synthetic route to vaccine glycoconjugates than their native counterparts. As shown in Table 2, olefination of **39** and **40** was uneventful and enamide esters **43** and **44** were obtained in 85% and 75% yields respectively, again as single isomers. Asymmetric hydrogenation, as before, also produced diastereomerically pure glycoamino acids **47** and **48** in excellent yields.

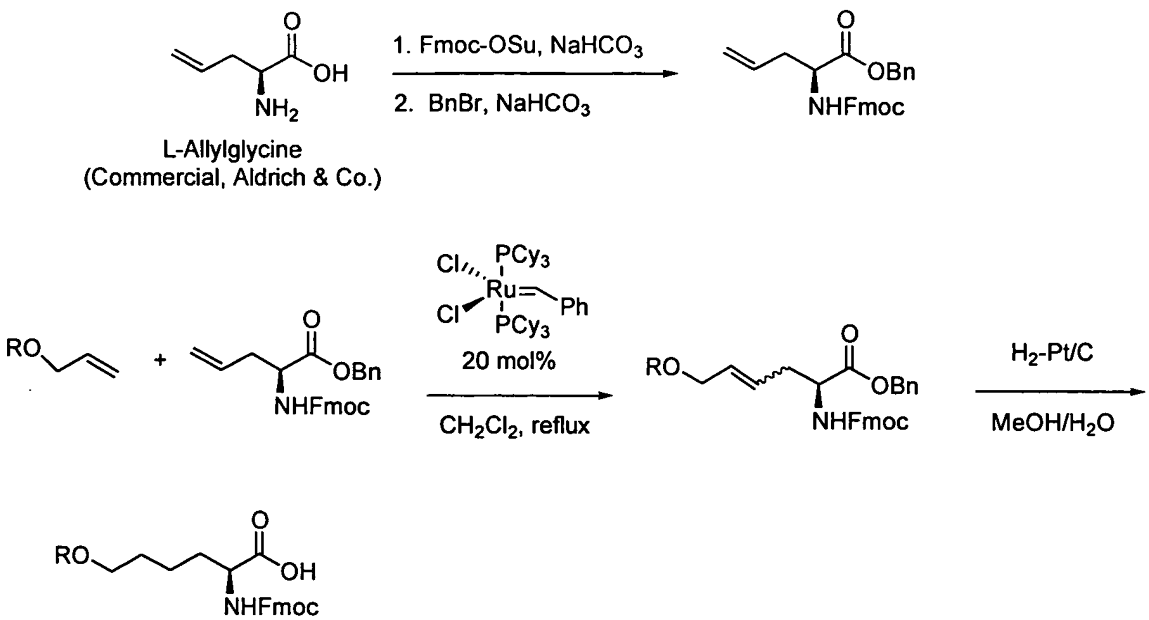
[0270] **Table 2:** Horner-Emmons olefination as applied to the preparation of glycoamino acids:



$\boxed{X}$ =	Conditions: (1) 1. O_3, CH_2Cl_2, MeOH, pyr.; then Me_2S; (2) TMG, **33**, THF, -78 ¡C product, yield	Conditions: (*S*, *S*)-Et-DuPHOS-Rh^+ H_2, 50 psi, THF product, yield, dr
 Globo-H **(37)**	**41**, 72%	**45**, 98%, >20:1
 Fucosyl GM_1 **(38)**	**42**,10-22%	**46**, 93%, >20:1
 Le^y **(39)**	**43**, 85%	**47**, 99%, >20:1
 **(40)** α-Tn antigen	**44**, 75%	**48**, 99%, >20:1

[0271] b. <u>Via Cross-Metathesis reaction of a suitable Allyl glycoside with a suitably protected allylglycine</u>

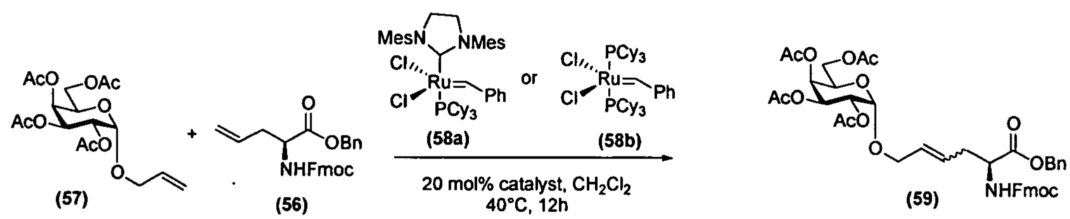
[0272] In certain other embodiments, as shown in Scheme 1 below, a tranformation utilizing a novel cross metathesis procedure may be used. The new approach involves a Ruthenium-catalyzed cross metathesis reaction of the allyl glycoside construct with a suitably protected allylglycine intermediate to give the corresponding eneamide ester adduct. Subsequent catalytic hydrogenation yields the desired diastereromerically pure glycoamino acid. R represents any carbohydrate domain described herein.



Scheme 1

[0273] Initial studies were carried out with peracetyl α-O-allyl-D-galactose **57** and Fmoc-L-allylglycine benzyl ester **56** (see Table 3).

[0274] **Table 3:** Cross-metathesis reaction conditions.

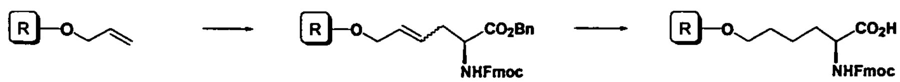


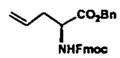
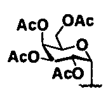
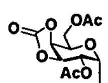
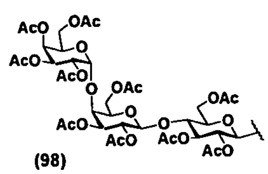
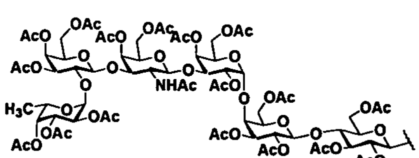
Entry	Equiv. of **56**	Catalyst	Yield (%)
1	2.0	**3**	26
2	2.0	**4**	49
3	5.0	**4**	70

[0275] Fmoc-L-allylglycine benzyl ester **56** was prepared by N-Protection of commercially available L-Allylglycine **55** as its Fmoc ester, followed by protection of the carboxylic moiety as its benzyl ester (see Figure 18). A two-fold excess of allylglycine **56**, in the presence of catalyst **58b** afforded the desired eneamide ester **59** in 26% yield, along with

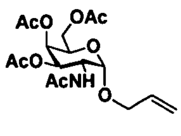
dimers of both substrates. When catalyst **58a** was used, the reaction proceeded in 49% yield, which was improved to 70% yield by increasing the excess of allylglycine **56** to 5 equivalents. These conditions also led to a marked decrease in dimerization of allylglycoside **57**, while increasing the yield of cross-metathesis reaction. Catalytic hydrogenation of **59** yields the desired galactose derived enamide ester substrate **60** in 87% yield as a single geometric isomer. As summarized in Table 4, the methodology was successfully applied to other glycosaccharides, including Globo-H allyl glycoside **37**.

[0276] **Table 4:** Cross-metathesis reaction as applied to the preparation of glycoamino acids:

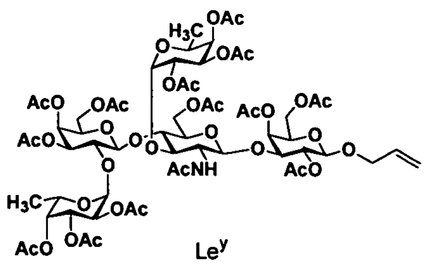


R =	Conditions:  20 mol% 58, CH_2Cl_2 40°C, 12 h product, yield	Conditions: H_2, 3% Pt/C $MeOH/H_2O$, 12 h product, yield
 (57)	59, 70%	60, 87%
 (95)	96, 66%	97, n/d
 (98)	99, 70%	100, n/d
 Globo-H (101)	102, 69%	103, 90%

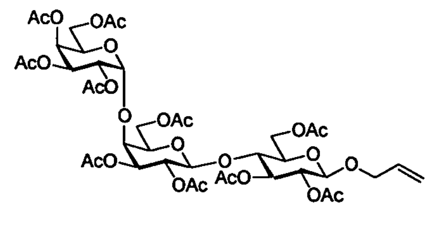
[0277] One of ordinary skill in the art will appreciate that this general methodology may be applied to a variety of other antigens of interest. Specifically, any carbohydrate domain available (or synthesized) as an O-allyl construct may be converted to the corresponding glycoaminoacid construct via the reaction steps described above. Examples of suitable glycosaccharide constructs include, but are not limited to:



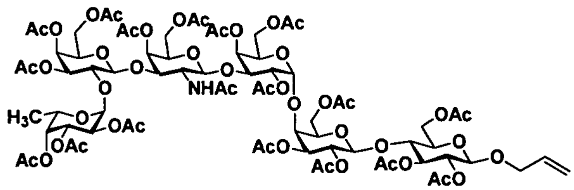
α-Tn antigen



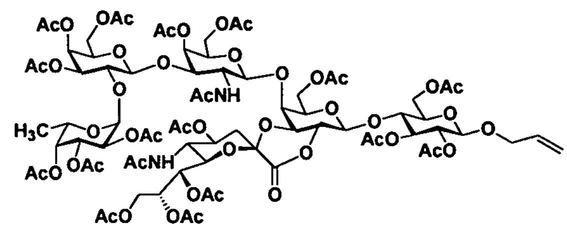
Le^y



Gb3 allyl glycoside



Globo-H



Fucosyl GM_1

[0278] With the glycoamino acids as described in a) and b) above in hand, it would thus be desirable to generate novel glycopeptides. Specifically, in one embodiment, a novel glycopeptide incorporating globo-H, Le^y, and Tn is provided. Specifically, the *C*-terminus is modified to include a conjugation handle for carrier protein KLH. The mercaptoacetamide unit has proven to be effective for this purpose. As shown in Figure 15, the Tn glycoamino acid **48** was treated with TBAF to reveal the corresponding carboxylic acid. Coupling with a di-amino spacer terminated in a protected mercaptoacetamide ($AcSCH_2C(O)(CH_2)_3NH_2$) under the agency of the BOP reagent (benzotriazol-1-oxytris(dimethylamino)phosphonium hexafluorophosphate) gave the corresponding amide in 50% yield for the 2 steps. Removal of the *N*-terminal Boc group gave amine **49** as its trifluoroacetate salt. The next antigen, Le^y, was prepared for coupling by reaction of **47** with TBAF to give acid **50**. Coupling of amine **49** with Le^y acid **50**, again with the BOP promoter, gave the Tn-Le^y di-peptide **51** in 86% yield. Lastly, Globo-H glycoamino acid **45** was treated with TBAF to give its corresponding acid **52**. Removal of the Boc protecting group in **51** followed by coupling with acid **52** gave the Tn-Le^y-Globo-H tri-peptide in 64% yield. Finally, the *N*-terminal Boc group was removed and the resulting anime

capped as its acetate to give tripeptide **53** in 95% yield . With all components in place, the ester protecting groups were removed with hydrazine in degassed methanol to give the fully deprotected glycopeptide **54** (Figure 16) in excellent yield. As discussed below, the inventive glycopeptides prepared as detailed herein can also be conjugated to a suitable carrier protein or lipid.

[0279] *2) Experimental* **General.** DuPHOS-Rh^+ catalysts were purchased from Strem Chemical Co., Newburyport, MA. All other commercial materials (purchased from Aldrich-Sigma) were used without further purification. The following solvents were obtained from a dry solvent system (passed through a column of alumina): THF, diethyl ether (Et_2O), CH_2Cl_2, toluene and benzene. All reactions were performed under an atmosphere of dry N_2, unless otherwise noted. NMR (^1H and ^{13}C) spectra were recorded on a Bruker AMX-400 MHz or Bruker Advance DRX-500 MHz and referenced to residual solvent unless otherwise noted. IR spectra were recorded with a Perkin-Elmer 1600 series-FTIR spectrometer and optical rotations were measured with a Jasco DIP-370 digital polarimeter using a 10-cm path length cell. Low-resolution mass spectral analysis were performed with a JOEL JMS-DX-303 HF mass spectrometer. Analytical TLC was performed on E. Merck silica gel 60 F254 plates and flash column chromatography was performed using the indicated solvents on E. Merck silica gel 60 (40-63 mm) or Sigma H-type silica gel (10-40 mm).

[0280] Procedure for the synthesis of 40 (as shown in Figure 17)

[0281] Trichloroacetimidate donor. The mixture of azidonitrates as shown in Figure 17 (1.66g, 4.41 mmol) was dissolved in CH_3CN (15 mL) and cooled to 0 °C. To the stirring solution was added Hunig's base (1.2 equiv., 0.925 mL) and benzene thiol (3.0 equiv., 1.35 mL). The reaction mixture was stirred at 0 °C for 1 hour and the ice bath was removed. After an additional 1 hour at room temperature, the reaction was concentrated under a stream of dry nitrogen. The resulting material was dissolved in a minimum amount of $CHCl_3$ and subjected to flash column chromatography (50% EtOAc/hexanes) to yield the hemiacetals (1.41, 97%). (Note 1: perform this flash in the hood, Note 2: isolate both anomers, which do separate on TLC/flash) The mixture of hemiacetals (1.41 g mg, 4.25 mmol) was dissolved in CH_2Cl_2 (8.5 mL) and trichloroacetonitrile (4.25 mL) was added, followed by K_2CO_3 (5.0 equiv., 2.93 g). The reaction stirred at room temperature overnight and was filtered through a plug of celite with additional methylene chloride. Concentration of the organic layer, followed by flash column

chromatography (10->25% EtOAc/hexanes) gave the β-trichloroacetimidate (1.30 mg, 77%) as a yellow oil. (Note: α-anomer elutes first, then the β-anomer)

[0282] **Pentenyl glycoside of α–Tn.** The TCA-donor as shown in Figure 17 (1.30 g, 2.72 mmol) was dissolved in THF (0.2M, 13.6 mL) and pentenyl alcohol (5.0 equiv., 1.2 mL) and cooled to -10 °C (acetone-ice bath). A portion of TMSOTf (0.1 equiv., 0.049 mL) was added and the reaction stirred for 1 hour. Solid $NaHCO_3$ was added and the reaction was filtered through celite, concentrated and subjected to flash column chromatography (25% EtOAc/hexanes). (Note 1: The diastereomeric anomers do not separate. Their ratio is determined by ^1H NMR. Note 2: The starting materials and products co-elute by TLC - gradient TLC (10% first, then 50%) can be used to visualize the progress of the reaction.) The isolated glycosides were taken up in 10 mL of AcSH and stirred at rt for 2 days. Evaporation of the solvent by a stream of dry nitrogen followed by flash column chromatography (5% acetone/toluene -> 10% acetone/toluene) gave 620 mg of the α-glycoside (55%) and an undertermined amount of β-glycoside. (Note: Ethyl acetate/hexanes mixtures will also separate anomers, but actone/toluene was determined to be superior.)

General procedure for olefination, 41. The preparation of enamide **41** (Globo-H) is representative of this procedure. The *n*-pentenyl glycoside **37** (58 mg, 0.0322 mmol) was dissolved in 10:10:1 MeOH:CH_2Cl_2:pyridine (3 mL, typically 0.05 M-0.01 M) and cooled to -78 °C. A stream of dry ozone was passed through the reaction mixture until a pale blue color persisted. The ozone source was removed and the reaction stirred at -78 °C for an additional 15 minutes, upon which time a stream of dry nitrogen was applied to remove excess ozone. Dimethyl sulfide (50 equivs., 0.118 mL) was added to the cooled mixture, the ice bath was removed and the reaction was allowed to stir at rt for 4 hours. The reaction was diluted with CH_2Cl_2 (10mL), washed with water (50mL), and back-extracted with additional CH_2Cl_2 (2x10 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated. The crude aldehyde was typically not purified, but was azeotroped dry with anhydrous benzene (3x3 mL) and used directly in the next step.

[0283] Phosphonate **33** (1.20 equivs., 14 mg) was dissolved in anhydrous THF (0.3 mL), cooled to -78 °C and tetramethyl guanidine (TMG) (1.25 equivs., 0.005 mL) was added dropwise. The reaction stirred at -78 for 30 minutes, followed by addition of the crude aldehyde

(0.0322 mmol) in additional THF (2x0.3 mL, typically 0.1-0.01 M total reaction volume). The reaction was allowed to stir to rt overnight (10-15h), was extracted with EtOAc (10mL), washed with 0.05 M aqueous HCl (50mL) and back-extracted with additional EtOAc (2x10 mL). (Note: All the TMG must be removed prior to asymmetric hydrogenation.) The combined organic layer was dried over $MgSO_4$, concentrated and purified by flash column chromatography (75% EtOAc/hexanes -> 100 % EtOAc) to yield the desired enamide ester **41** as a single isomer. 72%, white foam; R_f 0.85 (100% EtOAc); IR ($CDCl_3$ film) 3373, 2956, 2951, 1748, 1370, 1069 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.65 (d, 1H, J=6.4 Hz), 6.44 (m ,1H), 6.07 (bs, 1H), 5.56 (d, 1H, J=3.1 Hz), 5.44 (d, 1H, J=3.4 Hz), 5.37 (d, 1H, J=3.3 Hz), 5.27 (dd, 1H, J=10.9, 3.0 Hz), 5.22 (d, 1H, J=2.6 Hz), 5.20-5.17 (m, 2H), 5.15 (d, 1H, J=2.1 Hz), 5.13 (d, 1H, J=4.9 Hz), 5.09 (dd, 1H, J=10.7, 7.3 Hz), 5.03 (dd, 1H, J=11.1, 3.3 Hz), 4.96 (dd, 1H, J=9.6, 3.5 Hz), 4.92 (dd, 1H, J=11.2, 3.4 Hz), 4.85 (dd, 1H, J=9.6, 8.0 Hz), 4.73 (dd, 1H, J=10.9, 2.5 Hz), 4.50-4.38 (m, 6H), 4.34 (t, 1H, J=6.2 Hz), 4.26-4.21 (m, 3H), 4.16-4.02 (m, 8H), 3.98 (d, 1H, J=2.0 Hz), 3.94 (t, 1H, J=6.4 Hz), 3.86-3.72 (m, 6H), 3.60-3.57 (m, 1H), 3.48-3.46 (m, 1H), 2.94-2.89 (m, 1H), 2.17-2.14 (m, 1H), 2.11-2.08 (m, 1H), 2.04 (s, 3H), 2.038 (s, 3H), 2.033 (s, 6H), 1.99 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.967 (s, 3H), 1.962 (s, 3H), 1.95 (s, 3H), 1.92 (s, 9H), 1.89 (s, 3H), 1.857 (s, 3H), 1.854 (s, 3H), 1.78 (s, 3H), 1.63-1.59 (m, 2H), 1.34 (s, 9H), 1.04 (d, 3H, J=6.5 Hz), 0.93-0.90 (m, 2H), -0.06 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.20, 171.44, 170.94, 170.65, 170.52, 170.48, 170.44, 170.36, 170.29, 170.21, 170.17, 169.97, 169.63, 169.49, 169.31, 168.85, 164.78, 153.19, 134.70, 126.80, 102.35, 101.99, 101.26, 100.25, 99.12, 998.66, 94.21, 80.24, 76.88, 75.98, 73.61, 73.36, 73.08, 72.80, 72.56, 72.37, 71.81, 71.68, 71.46, 71.28, 70.78, 70.69, 70.67, 70.37, 70.06, 70.01, 68.92, 68.82, 67.99, 67.95, 67.54, 67.28, 66.94, 64.42, 62.14, 61.67, 61.29, 61.09, 60.92, 56.16, 28.12, 27.98, 24.52, 23.80, 23.03, 20.81, 20.73, 20.70, 20.68, 20.64, 20.60, 20.59, 20.54, 20.46, 20.40, 17.37, 17.24, 15.85, 15.48, 14.01, -1.58; HRMS (FAB) cald. for $C_{88}H_{128}N_2O_{51}SiNa$ 2079.7145, found 2079.7174.

[0284] **Lactose Enamide 34.** 88%, white foam; R_f 0.45 (66% EtOAc/hexanes); IR ($CDCl_3$ film) 3407, 3146, 2954, 2898, 1752, 1654, 1233, 1167, 1055 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.34 (m, 1H), 6.03 (bs, 1H), 5.22 (d, 1H, J=3.2 Hz), 5.07 (t, 1H, J=9.4 Hz), 4.99 (dd, 1H, J=10.3, 7.9 Hz), 4.83 (dd, 1H, J=10.5, 3.3 Hz), 4.77 (t, 1H, J=9.3 Hz), 4.37-4.33 (m, 3H), 4.20-4.11 (m, 3H), 4.08-4.00 (m, 3H), 3.82-3.65 (m, 5H), 3.49-3.46 (m, 1H), 3.39-3.34 (m, 1H), 2.16-

2.14 (m, 1H), 2.11-2.09 (m, 1H), 2.04 (s, 3H), 2.02 (s, 3H), 1.94 (s, 3H), 1.93 (s, 3H), 1.92 (s, 6H), 1.84 (s, 3H), 1.62-1.59 (m, 2H), 1.34 (s, 9H), 0.94-0.89 (m, 2H), 0.05 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.29, 170.21, 170.03, 169.94, 169.66, 169.50, 168.97, 164.77, 153.20, 134.70, 100.97, 100.31, 80.23, 76.17, 72.69, 72.51, 71.56, 70.87, 70.52, 68.95, 68.83, 66.47, 63.54, 61.88, 60.66, 33.81, 28.05, 27.92, 24.47, 20.73, 20.68, 20.57, 20.51, 20.39, 17.21, -1.60; HRMS (FAB) cald. for $C_{43}H_{67}NO_{21}SiNa$ 986.4013, found 986.4029.

[0285] **Lewis^y enamide 43**. 85%, white foam; R_f 0.45 (75% EtOAc/hexanes); IR ($CDCl_3$ film) 3371, 2965, 2956, 1746, 1371, 1231, 1069 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.94 (d, 2H, J=7.2 Hz), 7.48 (t, 1H), 7.36 (t, 2H), 6.21 (t, 1H), 5.71 (m, 1H), 5.38 (d, 1H, J=3.2 Hz), 5.29 (dd, 1H, J=10.8, 8.8 Hz), 5.22-5.19 (m, 4H), 5.05-4.78 (m, 10H), 4.43 (dd, 1H, J=14.1, 8.0 Hz), 4.36 (dd, 1H, J=9.7, 5.1 Hz), 4.27 (m, 1H), 4.15-4.30 (m, 5H), 3.86 (dd, 1H, J=10.1, 3.3 Hz), 3.78-3.71 (m, 3H), 3.62 (dd, 1H, J=9.8, 1.7 Hz), 3.30 (d, 1H, J=9.6 Hz), 3.18-3.08 (m, 1H), 3.04 (bm, 1H), 2.08 (s, 3H), 2.04 (s, 6H), 2.01 (s, 3H), 1.99 (s, 9H), 1.98 (s, 3H), 1.94 (s, 3H), 1.89 (s, 3H), 1.88 (s, 3H), 1.86 (s, 3H), 1.83 (s, 3H), 1.55-1.52 (m, 2H), 1.32 (s, 9H), 1.01-1.00 (m, 6H), 0.91-0.88 (m, 2H), -0.07 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.56, 172.40, 172.28, 172.11, 172.06, 172.04, 171.87, 171.77, 171.57, 171.43, 171.20, 171.06, 166.69, 166.32, 154.76, 136.48, 135.14, 131.29, 130.76, 130.19, 127.93, 102.82, 101.83, 101.72, 97.65, 97.11, 81.66, 75.40, 74.80, 74.55, 74.32, 74.01, 72.87, 72.65, 72.35, 72.28, 71.14, 70.66, 69.73, 69.28, 69.15, 69.02, 68.34, 66.43, 65.38, 64.92, 63.36, 62.05, 61.80, 59.87, 29.66, 29.62, 29.48, 25.83, 24.02, 22.51, 22.46, 22.31, 22.14, 22.12, 22.09, 22.0o3, 18.75, 17.30, 17.00, 15.63, -0.04; HRMS cald. for $C_{79}H_{112}N_2O_{41}SiNa$, found x.

[0286] **Tn enamide 44**. 75%, white foam; R_f 0.80 (100% EtOAc); IR ($CDCl_3$ film) 3340, 3071, 2954, 1715, 1663, 1498, 1369, 1218, 1162, 1049 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.38 (bs, 1H), 6.15 (bs, 1H), 5.26 (d, 1H, J=2.7 Hz), 5.02 (dd, 1H, J=11.5, 3.2 Hz), 4.79 (s, 1H), 4.48-4.42 (m, 1H), 4.16-4.12 (m, 2H), 4.03 (m, 1H), 3.99-3.92 (m, 2H), 3.62-3.56 (m, 1H), 3.40-3.35 (m, 1H), 2.26-2.16 (m, 2H), 2.03 (s, 3H), 1.92 (s, 3H), 1.86 (s, 3H), 1.84 (s, 3H), 1.67 (s, 3H), 1.33 (s, 9H), 0.94-0.9 (m, 2H), -0.06 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.28, 170.11, 170.06, 164.70, 134.6, 108.73, 97.26, 80.31, 67.95, 67.05, 66.35, 63.51, 61.67, 47.46, 27.87, 27.49, 22.78, 20.43, 17.05, -1.74; HRMS (FAB) cald. for $C_{30}H_{51}N_2O_{13}SiNa$ 675.3160, found 675.3124.

[0287] **Fucosyl GM$_1$ enamide 42.** 10-22%; R_f 0.25 (10% MeOH/EtOAc); ^{1}H NMR (MeOH, 500 MHz) δ 7.94 (d, 2H, J=7.2 Hz), 7.48 (t, 1H), 7.36 (t, 2H), 6.21 (t, 1H), 5.71 (m, 1H), 5.38 (d, 1H, J=3.2 Hz), 5.29 (dd, 1H, J=10.8, 8.8 Hz), 5.22-5.19 (m, 4H), 5.05-4.78 (m, 10H), 4.43 (dd, 1H, J=14.1, 8.0 Hz), 4.36 (dd, 1H, J=9.7, 5.1 Hz), 4.27 (m, 1H), 4.15-4.03 (m, 5H), 3.86 (dd, 1H, J=10.1, 3.3 Hz), 3.78-3.71 (m, 3H), 3.62 (dd, 1H, J=9.8, 1.7 Hz), 3.39-3.37 (m, 1H), 3.30 (bd, 1H, J=9.6 Hz), 3.18-3.08 (m, 1H), 3.04 (bm, 1H), 2.08 (s, 3H), 2.04 (s, 6H), 2.01 (s, 3H), 1.99 (s, 9H), 1.98 (s, 3H), 1.94 (s, 3H), 1.89 (s, 3H), 1.88 (s, 3H), 1.86 (s, 3H), 1.83 (s, 3H), 1.5-1.52 (m, 2H), 1.32 (s, 9H), 1.01-1.00 (m, 6H), 0.91-0.88 (m, 2H), -0.07 (s, 9H).

[0288] **General procedure for asymmetric hydrogenation.** Under an inert deoxygenated atmosphere, $[(COD)Rh\text{-}((S,S)\text{-Et-DuPHOS})]^+OTf^-$ (0.005 mmol, 5 mol%) and the desired enamide ester (0.100 mmol) were dissolved in deoxygenated anhydrous THF (10 mL, 0.01 M) in a Fischer-Porter tube. The reaction vessel was pressurized with 50 psi of H_2 after three vacuum/H_2 cycles and stirred at 25 °C for 24-36 hours, or until the reaction turned from light orange to brown in color. The vessel was depressurized, the mixture concentrated and purified through a short plug of silica gel to yield the glycoamino acid.

[0289] **Lactose glycoamino acid 35.** 98%; R_f 0.45 (66% EtOAc/hexanes); ^{1}H NMR (C_6D_6, 500 MHz) δ 5.54 (dd, 1H, J=10.4, 8.0 Hz), 5.48 (d, 1H, J=3.2 Hz), 5.39 (t, 1H, J=9.2 Hz), 5.21 (dd, 1H, J=6.2, 1.1 Hz), 5.12 (d, 1H, J=3.1 Hz), 5.09 (d, 1H, J=3.3 Hz), 4.54-4.51 (m, 2H), 4.33 (d, 1H, J=7.8 Hz), 4.19-4.06 (m, 6H), 3.74-3.58 (m, 2H), 3.49-3.40 (m, 1H), 3.38 (d, 1H, J=10.9 Hz), 3.23-3.16 (m, 2H), 1.96 (s, 3H), 1.94 (s, 3H), 1.90 (s, 3H), 1.74 (s, 3H), 1.73 (s, 3H), 1.64 (s, 3H), 1.51 (s, 3H), 1.45 (s, 9H), 0.91-0.88 (m, 2H), -0.10 (s, 9H); ^{13}C NMR (C_6D_6, 100 MHz) δ 173.26, 170.44, 170.41, 170.18, 170.09, 169.35, 156.05, 102.98, 101.06, 79.73, 77.59, 74.13, 73.21, 72.73, 71.94, 71.19, 70.10, 69.58, 67.28, 63.76, 63.01, 61.25, 54.41, 34.76, 32.76, 28.62, 28.80, 25.75, 22.45, 21.18, 20.93, 20.84, 20.76, 20.57, 20.46, 20.15, 17.83, -1.29; HRMS (FAB) cald. for $C_{42}H_{67}NO_{22}SiNa$ 988.3870, found 988.3821.

[0290] **Globo-H glycoamino acid 45.** 98%; IR ($CDCl_3$ film) 3373, 2956, 2951, 1748, 1370, 1069 cm^{-1}; ^{1}H NMR (C_6D_6, 500 MHz) δ 6.54 (d, 1H, J=6.5 Hz), 5.89 (d, 1H, J=3.5 Hz), 5.86 (d, 1H, J=3.1 Hz), 5.74-5.69 (m, 3H), 5.50-5.46 (m, 2H), 5.39-5.34 (m, 2H), 5.31 (dd, 1H, J=13.4, 0.7 Hz), 5.26 -5.19 (m, 2H), 5.15 (d, 1H, J=8.1 Hz), 5.09-5.06 (m, 3H), 4.82 (dd, 1H, J=10.9, 2.5 Hz), 4.74-4.72 (m, 1H), 4.70-4.69 (m, 1H), 4.66 (t, 1H), 4.58-4.49 (m, 3H), 4.45-4.41

(m, 2H), 4.37-4.33 (m, 2H), 4.24-4.22 (m, 2H), 4.20-4.12 (m, 3H), 4.04-4.01 (m, 2H), 3.91-3.86 (m, 3H), 3.71-3.69 (m, 2H), 3.64-3.57 (m, 2H), 4.43 (t, 1H) , 3.28-3.27 (m, 1H), 3.23-3.21 (m, 1H), 2.38 (s, 3H), 2.30 (s, 3H), 2.19 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.93 (s, 3H), 1.92 (s, 3H), 1.90 (s, 3H), 1.89 (s, 3H), 1.88 (s, 3H), 1.84 (s, 3H), 1.82 (s, 3H), 1.75 (s, 3H), 1.73 (s, 3H), 1.71 (s, 3H), 1.68 (s, 3H), 1.64 (s, 3H), 1.61 (s, 3H), 1.46 (s, 3H), 1.45 (s, 9H), 1.26-1.22 (m, 2H), 1.08 (d, 3H, J=6.5 Hz), 0.89 (t, 2H), -0.10 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.87, 172.28, 171.52, 170.77, 170.61, 170.56, 170.45, 170.40, 170.29, 170.07, 169.75, 169.58, 169.44, 168.95, 155.38, 102.00, 101.29, 100.38, 99.21, 98.77, 94.31, 73.65, 73.34, 73.10, 72.61, 72.38, 71.84, 71.65, 71.58, 71.30, 70.81, 70.68, 70.17, 70.06, 69.59, 69.09, 68.85, 68.05, 67.99, 67.56, 67.30, 64.46, 63.64, 62.16, 61.74, 61.35, 61.12, 60.96, 56.11, 53.45, 32.30, 29.65, 28.96, 28.29, 28.10, 23.11, 21.69, 20.88, 20.85, 20.80, 20.76, 20.72, 20.67, 20.62, 20.55, 20.48, 17.31, 15.88, -1.55; HRMS (FAB) cald. for $C_{88}H_{130}N_2O_{51}SiNa$ 2081.7302, found 2081.7247.

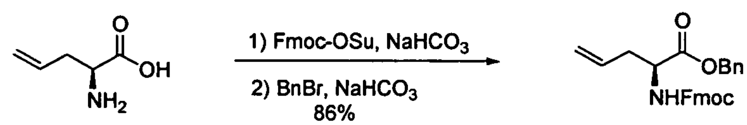
[0291] **Lewis^y glycoamino acid 42**. 99%; IR ($CDCl_3$ film) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.24 (d, 2H, J=8.4 Hz), 7.27-7.18 (m, 3H), 5.83 (dd, 1H, J=10.1, 8.0), 5.78 (d, 1H, J=3.2 Hz), 5.76 (d, 1H, J=3.0 Hz), 5.74-5.71 (m, 2H), 5.69 (d, 1H, J=3.2 Hz), 5.66 (d, 1H, J=3.3Hz), 5.55 (d, 1H, J=3.3 Hz), 5.50 (m, 1H), 5.47 (d, 1H, J=3.8 Hz), 5.41-5.27 (m, 5H), 4.93 (d, 1H, J=7.8 Hz), 4.86 (d, 1H, J=8.6 Hz), 4.77-4.70 (m, 2H), 4.62 (d, 1H, J=7.8 Hz), 4.56-4.53 (m, 1H), 4.47-4.35 (m, 5H), 4.34-4.25 (m, 4H), 4.11-4.00 (m, 5H), 3.92-3.89 (m, 1H), 3.75-3.65 (m, 3H), 3.64-3.61 (t, 1H), 3.28-2.24 (m, 1H), 2.22 (s, 3H), 2.21 (s, 3H), 1.93 (s, 3H), 1.90 (s, 3H), 1.82 (s, 3H), 1.78 (s, 3H), 1.73 (s, 3H), 1.72 (s, 3H), 1.66 (s, 3H), 1.65 (s, 3H), 1.64 (s, 3H), 1.63 (s, 3H), 1.51 (s, 3H), 1.50 (d, 3H, J=6.5 Hz), 1.46 (s, 9H), 1.38 (d, 3H, J=6.5 Hz), 1.33-1.27 (m, 2H), 1.22-1.21 (m, 2H), 0.91-0.80 (m, 4H), -0.12 (s, 9H); ^{13}C NMR (C_6D_6, 100 MHz) δ 172.86, 171.05, 170.59, 170.52, 170.46, 170.37, 170.26, 170.01, 169.97, 169.90, 165.44, 155.61, 133.24, 130.40, 101.59, 100.98, 100.71, 97.09, 96.21, 79.22, 76.42, 74.77, 74.04, 73.69, 72.03, 71.65, 71.14, 70.14, 69.20, 68.66, 68.55, 68.20, 67.99, 67.25, 65.67, 64.56, 63.23, 62.41, 61.33, 60.94, 58.23, 53.84, 39.12, 32.25, 29.30, 28.41, 22.88, 22.01, 20.93, 20.66, 20.58, 20.47, 20.40, 20.18, 20.02, 17.40, 16.36, 15.94, -1.62; HRMS (FAB) cald. for $C_{79}H_{114}N_2O_{41}SiNa$ 1797.6558, found 1797.6528.

[0292] **Tn glycoamino acid 43**. 99%; IR ($CDCl_3$ film) 3362, 2954, 2990, 2871, 1749, 1716, 1683, 1668, 1520, 1369, 1249, 1164, 1047 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.66 (d,

1H, J=9.3 Hz), 5.24 (d, 1H, J=2.8 Hz), 5.03 (dd, 1H, J=11.4, 3.3 Hz), 4.98 (d, 1H, J=8.1), 4.73 (d, 1H, J=3.3 Hz), 4.48-4.42 (m, 1H), 4.17-4.07 (m, 3H), 4.05-3.93 (m, 3H), 3.59-3.54 (m, 1H), 3.33-3.27 (m, 1H), 2.04 (s, 1H), 1.93 (s, 3H), 1.87 (s, 3H), 1.86 (s, 3H), 1.73-1.70 (m, 1H), 1.57-.150 (m, 3H), 1.31 (s, 9H), 0.91-0.87 (m, 2H), -0.06 (s, 9H); ^{13}C NMR (C_6D_6, 100 MHz) δ 173.44, 171.04, 170.57, 170.35, 169.89, 156.06, 98.59, 79.82, 69.29, 68.30, 68.07, 67.43, 64.01, 62.33, 54.33, 48.63, 32.87, 29.05, 28.76, 23.24, 22.82, 20.89, 20.66, 20.47, 17.85, -1.28; HRMS (FAB) cald. for $C_{30}H_{53}N_2O_{13}SiNa$ 677.3316, found 677.3352.

[0293] **General procedure for cross-metathesis reaction.** The preparation of enamide **60** (galactose) is representative of this procedure.

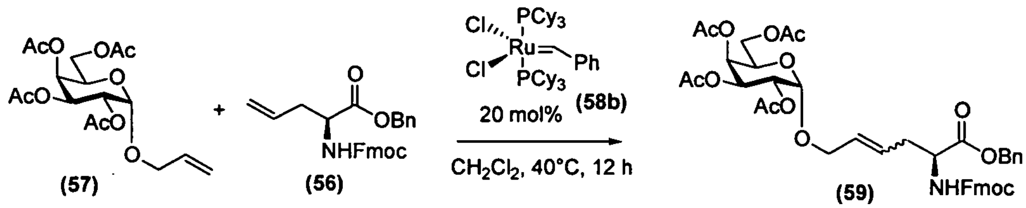
[0294] Step 1: Protection of L-allylglycine



(55) (56)

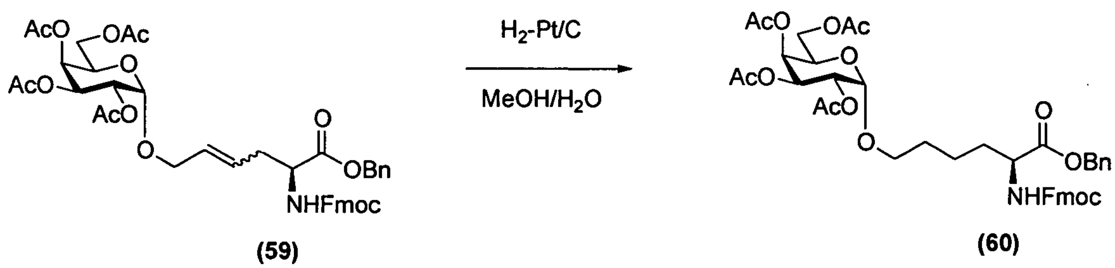
[0295] L-allylglycine (**55**, 2.5 g, 21.7 mmol) and Fmoc-*O*-succinimide (8.6 g, 25.5 mmol, 1.2 equiv) were mixed in 200 mL acetone:water (3:1) mixture and treated with solid $NaHCO_3$ (18.2 g, 217 mmol, 10.0 equiv). The reaction was stirred at rt for 24 h, diluted with EtOAc (200 mL) and washed with 1N HCl (300 mL) and brine (300 mL). The organic layer was dried ($MgSO_4$) and concentrated. The crude carbamate was dissolved in DMF (100 mL) and treated with $NaHCO_3$ (5.5 g, 65.1 mmol, 3.0 equiv) and benzyl bromide (25.4 mL, 217 mmol, 10.0 equiv). The reaction mixture was stirred at rt for 24 h, diluted with EtOAc (150 mL) and washed with water (2×200 mL) and brine (200 mL). The organic layer was dried ($MgSO_4$) and purified by silica gel chromatography (12-20% EtOAc/hexane) affording 7.9 g (86%) of **55** as a white solid: ^1H NMR (400 MHz) δ 7.77 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 7.5 Hz, 2H), 7.43 - 7.29 (m, 9H), 5.71 - 5.62 (m, 1H), 5.34 (d, J = 8.3 Hz, 1H), 5.20 (ABq, J = 8.2 Hz, 2H), 5.15 - 5.08 (m, 2H), 4.56 - 4.53 (m, 1H), 4.39 (d, J = 7.2 Hz, 2H), 4.23 (t, J = 7.2 Hz, 1H), 2.62 - 2.50 (m, 2H); LRMS (ESI) $M_{calc.}$ 427.1 for $C_{27}H_{25}NO_4$, $(M+Na)_{found}$ 450.1.

[0296] Step 2: Cross metathesis reaction:



[0297] Peracetylated *O*-allyl-D-galactose (**57**, 185 mg, 0.47 mmol) was mixed with Fmoc-L-allylglycine-OBn (**56**, 1.01 g, 2.38 mmol, 5.0 equiv) in CH_2Cl_2 (3 mL) and treated with bis(tricyclohexylphosphine) ruthenium Grubbs catalyst **58** (39 mg, 0.047 mmol, 0.1 equiv). The reaction was heated to reflux. After 6h, another 0.1 equiv of catalyst was further added. The reaction was cooled to rt after 12 h (total) and purified by silica gel chromatography (20-28-32-40% EtOAc/hexanes) to afford metathesis product **59** (250 mg, 70 %), along with 392 mg (39% of 5.0 equiv) recovered **58**.

[0298] Step 3: Catalytic hydrogenation:



[0299] The *E*:*Z* mixture **59** (26 mg, 0.03 mmol) was dissolved in 1.1 mL methanol:water (10:1) and treated with 3% Pt/C (26 mg). The reaction flask was stirred under a hydrogen balloon for 12 h, followed by filtration to remove the solid catalyst. The filtrate was concentrated and purified by silica gel chromatography (64% EtOAc/hexane, with 1% AcOH) to afford amino acid **60** (20 mg, 87%) as a white solid: ^{1}H NMR (400 MHz) □ 7.77 (d, J = 7.5 Hz, 2H), 7.61 (d, J = 7.5 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.31 (t, J = 7.5 Hz, 2H), 5.56 (d, J = 8.1 Hz, 1H), 5.45 (d, J = 3.0 Hz, 1H), 5.34 (dd, J = 10.0, 3.0 Hz, 1H), 5.21 - 5.10 (m, 2H), 4.43 - 4.38 (m, 3H), 4.25 - 4.16 (m, 3H), 4.25 - 4.00 (m, 1H), 3.79 - 3.76 (m, 1H), 3.47 - 3.44 (m, 1H), 2.16 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.89 - 1.86 (m, 2H), 1.67 - 1.65 (m, 2H), 1.55 - 1.48 (m, 2H); LRMS (ESI) $M_{calc.}$ 699.2 for $C_{35}H_{41}NO_{14}$, $(M+Na)_{found}$ 722.3.

[0300] **Globo-H glycoamino acid 103**

[0301] Peracetylated Globo-H allyl glycoside (20 mg, 0.011 mmol, Ref: Park, T.K.; Kim, I.J.; Hu, S.; Bilodeau, M.T.; Randolph, J.T.; Kwon, O.; Danishefsky, S.J. *J. Am. Chem. Soc.* **1996**, *118*, 11488-11500) and Fmoc-L-allylglycine benzyl ester (24 mg, 0.056 mmol, 5.0 equiv) in methylene chloride (0.05 mL) were treated bis(tricyclohexylphosphine) ruthenium Grubbs catalyst (2 mg, 0.001 mmol, 0.1 equiv). The reaction was heated to reflux. After 6h, another 0.1 equiv of catalyst was further added. The reaction was cooled to rt after 12 h (total) and purified by silica gel chromatography (80-90% EtOAc/hexanes) to afford metathesis product (16 mg, 69 %). The *E*:*Z* mixture (20 mg, 0.009 mmol) was dissolved in 0.55 mL methanol:water (10:1) and treated with 3% Pt/C (20 mg). The reaction flask was stirred under a hydrogen balloon for 12 h, followed by filtration to remove the solid catalyst. The filtrate was concentrated and purified by silica gel chromatography (EtOAc - 1% AcOH/EtOAc) to afford Globo-H amino acid (17 mg, 90%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 7.5 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.31 (t, J = 7.5 Hz, 2H), 6.77 (d, J = 8.0 Hz, 1H), 5.59 (d, J = 2.7 Hz, 1H), 5.54 (d, J = 10.0 Hz, 1H), 5.47 (d, J = 3.0 Hz, 1H), 5.40 (d, J = 3.0 Hz, 1H), 5.30 (dd, J = 11.0, 3.1 Hz, 1H), 5.27 - 5.20 (m, 3H), 5.05 – 4.98 (m, 2H), 4.94 (dd, J = 10.5, 3.1 Hz, 1H), 4.87 (t, J = 8.5 Hz, 1H), 4.76 (dd, J = 10.1, 2.3 Hz, 1H), 4.60 - 4.34 (m, 10H), 4.35 - 4.23 (m, 2H), 4.20 - 3.94 (m, 11H), 3.93 - 3.72 (m, 5H), 3.69 - 3.62 (m, 1H), 3.58 – 3.52 (m, 1H), 3.21 – 3.06 (m, 1H), 2.19 – 2.14 (m, 12H), 2.12 (s, 3H), 2.11 – 2.02 (m, 27H), 2.01 (s, 3H), 1.99 – 1.95 (m, 6H), 1.93 (s, 3H), 1.82 - 1.68 (m, 2H), 1.67 - 1.56 (m, 2H), 1.53 - 1.40 (m, 2H), 1.15 (d, J = 6.2 Hz, 3H); LRMS (ESI) $M_{calc.}$ 2080.6 for $C_{93}H_{120}N_2O_{51}$, $(M+H)_{found}$ 2081.9, $(M+Na)_{found}$ 2103.7.

[0302] **General procedure for *N*-Boc deprotection.** The desired glycoamino acid (0.100 mmol) was dissolved in CH_2Cl_2 (3.0mL) with stirring. Trifluoroacetic acid (TFA) (3.0mL) was added dropwise and the reaction stirred at rt for 1 hour. The mixture was then concentrated with a stream of dry N_2 and azeotroped with anhydrous benzene (2x5mL) to give the crude amine as its TFA salt which was typically used without further purification.

[0303] **General procedure for TSE ester deprotection.** The desired glycoamino acid (0.100 mmol) was dissolved in THF (1.0-3.0 mL) and cooled to 0 °C. A 1.0 M solution of TBAF in THF (0.250 mmol, 2.5 equivs.) was added dropwise, the ice bath removed and the

reaction stirred at rt for 1-2 hour, as judged by TLC. (Note: prolonged reaction times, *i.e.* >10 h, may result in deacetylation.) The reaction mixture was diluted with CH_2Cl_2 (10mL), washed with 0.05M aqueous HCL (50 mL), and back-extracted with additional CH_2Cl_2 (2x10 mL). The combined organic layer was dried over anhydrous Mg_2SO_4 and concentrated. The crude acid was typically used without further purification. **Acid 36**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.22 (d, 1H, J=2.8 Hz), 5.07 (t, 1H, J=9.3 Hz), 4.98 (dd, 1H, J=10.4, 5.9 Hz), 4.84 (dd, 1H, J=10.4, 3.5 Hz), 4.75 (dd, 1H, J=9.5, 8.0 Hz), 4.42-4.35 (m, 2H), 4.34-4.31 (m, 1H), 4.15-4.14 (m, 1H), 4.03-3.94 (m, 4H), 3.77-3.65 (m, 5H), 3.49-3.45 (m, 1H), 3.37-3.33 (m, 1H), 3.10-3.07 (m, 1H), 2.03 (s, 3H), 2.00 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.92 (s, 3H), 1.84 (s, 3H), 1.51-1.46 (m, 4H), 1.32 (s, 9H).

[0304] **General procedure for BOP reagent promoted peptide coupling.** The desired amine and acid (equimolar amounts) were azeotroped together with anhydrous benzene and dried under high vacuum. The mixture was dissolved in CH_2Cl_2 (0.1-0.05M), BOP reagent (1.25 equivs) was added and the solution cooled to 0 °C over 15 minutes. A dropwise addition of Hunig's base (15 equivs.) was followed by removal of the ice bath. The reaction stirred at rt for 2-4 h, as judged by TLC. Concentration of the reaction mixture was followed by purification by flash column chromatography. In cases where bi-product HMPA was difficult to remove, the peptide was subjected to sephadex purification (LH-20, MeOH).

[0305] ***N*-Boc Tn with mercatoacetamide spacer.** 54%, colorless oil; R_f 0.35 (10% MeOH/EtOAc); IR ($CDCl_3$ film) 3303, 3078, 2974, 2935, 2872, 1748, 1703, 1692, 1658, 1535, 1440, 1369, 1245, 1166 cm^{-1}; ^{1}H NMR (MeOH, 400 MHz) δ 5.40 (d, 1H, J=2.9 Hz), 5.13 (dd, 1H, J=11.6, 3.2 Hz), 4.42 (dd, 1H, J=11.5, 3.5 Hz), 4.23 (t, 1H, J=6.7 Hz), 4.14-4.05 (m, 2H), 3.96 (m, 1H), 3.74-3.69 (m, 1H), 3.60 (s, 2H), 3.49-4.44 (m, 1H), 3.26-3.13 (m, 5H), 2.36 (s, 3H), 2.12 (s, 3H), 2.02 (s, 3H), 1.94 (s, 3H), 1.93 (s, 3H), 1.67-1.59 (m, 6H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 195.59, 172.53, 170.78, 170.54, 170.39, 170.30, 168.51, 155.62, 97.59, 79.95, 68.29, 67.96, 67.26, 66.38, 61.83, 60.28, 54.58, 47.62, 36.47, 35.97, 33.02, 31.92, 30.18, 29.14, 28.37, 28.17, 23.07, 22.35, 20.68, 20.64; HRMS (MALDI) cald. for $C_{32}H_{52}N_4O_{14}SNa$ 771.3093, found 771.3070.

[0306] **Le^{Y}/Tn dipeptide 51.** 86%, white film; R_f 0.65 (20% MeOH/EtOAc); ^{1}H NMR (MeOH, 400 MHz) δ 8.06 (d, 2H, J=7.4 Hz), 7.63 (t, 1H), 7.51 (t, 2H), 5.55 (d, 1H, J=3.3 Hz), 5.40 (d, 1H, 2.7 Hz), 5.38 (d, 1H, J=2.7 Hz), 5.32 (d, 1H, J=3.3 Hz), 5.29 (d, 1H, J=4.1 Hz), 5.26 (d, 1H), 5.21-5.12 (m, 5H), 5.01 (q, 1H), 4.93 (m, 2H), 4.90 (m, 2H), 4.79 (d, 1H, J=10.8 Hz), 4.71 (d, 1H), 4.64 (d, 1H), 4.50-4.01 (m, 15H), 3.88-3.60 (m, 7H), 3.60 (s, 2H), 3.51-3.42 (m, 2H), 3.20-3.13 (m, 5H), 2.36 (s, 3H), 2.20 (s, 3H), 2.16 (s, 3H), 2.15 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.02 (s, 6H), 1.97 (s, 6H), 1.95 (s, 3H), 1.94 (s, 9H), 1.91 (s, 3H), 1.68-1.63 (m, 8H), 1.44 (s, 9H), 1.15 (d, 3H, J=6.3 Hz), 1.14 (d, 3H, J=6.3 Hz); ^{13}C NMR (MeOH, 100 MHz) δ 196.26, 175.39, 174.30, 173.75, 173.22, 172.80, 172.72, 172.56, 172.51, 172.44, 172.39, 172.30, 172.19, 172.12, 171.85, 171.75, 171.64, 170.76, 166.77, 158.24, 134.92, 131.29, 131.10, 129.99, 103.53, 102.80, 101.77, 99.12, 97.81, 97.30, 80.90, 78.50, 75.88, 75.11, 74.65, 74.32, 73.01, 72.70, 72.56, 72.47, 72.18, 71.91, 70.86, 69.84, 69.62, 69.42, 69.09, 68.90, 67.84, 66.43, 65.43, 63.50, 63.21, 62.57, 61.68, 56.42, 55.96, 54.89, 38.21, 37.74, 37.12, 33.89, 32.72, 30.42, 30.21, 30.01, 28.92, 23.72, 23.44, 22.93, 22.73, 21.32, 21.13, 20.97, 20.86, 20.74, 20.60, 16.67, 16.29; HRMS (MALDI) cald. for $C_{101}H_{144}N_6O_{52}SiNa$ 2327.8421, found 2327.8536.

[0307] ***N*-Boc Globo-H/Le^{y}/Tn tripeptide**. 64%, white film; R_f 0.45 (10% MeOH/EtOAc); ^{1}H NMR (MeOH, 400 MHz) δ 8.05 (d, 2H, J=7.4 Hz), 7.63 (t, 1H), 7.50 (t, 2H), 5.64 (d, 1H, J=2.8 Hz), 5.55 (d, 1H, J=3.6 Hz), 5.43 (d, 1H, J=3.2 Hz), 5.40 (d, 1H, J=2.4 Hz), 5.37 (d, 1H, J=2.5 Hz), 5.32-5.25 (m, 7H), 5.23-5.12 (m, 10H), 5.08-5.05 (m, 2H), 5.00 (d, 1H, J=7.5 Hz), 4.96 (d, 1H, J=3.1 Hz), 4.94 (m, 2H), 4.90 (m, 2H), 4.86 (m, 2H), 4.82-4.77 (m, 3H), 4.72-4.70 (m, 3H), 4.64-4.59 (m, 3H), 4.51-4.35 (m, 9H), 4.32-3.92 (m, 31H), 3.86-3.67 (m, 13H), 3.60 (s, 2H), 3.59 (m, 1H), 3.51 (m, 1H), 3.47-3.44 (m, 2H), 3.24-3.18 (m, 5H), 2.36 (s, 3H), 2.19 (s, 3H), 2.17 (s, 3H), 2.16 (s, 3H), 2.15 (s, 3H), 2.14 (s, 3H), 2.138 (s, 3H), 2.133 (s, 3H), 2.12 (s, 3H), 2.116 (s, 3H), 2.115 (s, 3H), 2.10 (s, 3H), 2.096 (s, 3H), 2.090 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.05 (s, 6H), 2.04 (s, 3H), 2.039 (s, 3H), 2.031 (s, 9H), 2.02 (s, 6H), 2.00 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.94 (s, 18H), 1.93 (s, 3H), 1.91 (s, 3H), 1.86 (s, 3H), 1.67-1.52 (m, 16H), 1.43 (s, 9H), 1.32 (d, 3H), 1.16-1.13 (m, 6H).

[0308] ***N*-Ac capped Globo-H/Le^y/Tn tripeptide 53.** 95%, white film; R_f 0.35 (10% MeOH/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.00 (d, 2H, J=7.3 Hz), 7.59 (t, 1H), 7.47 (t, 2H), 6.70 (d, 1H, J=6.3 Hz), 6.61 (d, 1H, J=9.8 Hz), 5.56 (d, 1H, J=2.9 Hz), 5.47 (d, 1H, J=2.1 Hz), 5.44 (d, 1H, J=3.4 Hz), 5.37 (d, 1H, J=3.2 Hz), 5.34 (d, 1H, J=2.7 Hz), 5.32-5.28 (m, 7H), 5.28 (d, 1H), 5.22-5.19 (m, 3H), 5.15-5.10 (m, 6H), 5.08 (m, 1H), 5.06 (m, 1H), 5.01-4.87 (m, 13H), 4.85-4.82 (m, 1H), 4.55-4.35 (m, 15H), 4.26-4.23 (m, 4H), 4.15-4.02 (m, 20H), 3.98-3.93 (m, 5H), 3.88-3.72 (m, 12H), 3.66 (m, 1H), 3.59-3.58 (m, 1H), 3.52 (s, 2H), 3.49-3.38 (m, 5H), 3.20 (m, 6H), 3.02-2.98 (m, 1H), 2.37 (s, 3H), 2.17 (s, 3H), 2.139 (s, 6H), 2.133 (s, 6H), 2.12 (s, 6H), 2.11 (s, 3H), 2.10 (s, 3H), 2.08 (s, 12H), 2.07 (s, 3H), 2.06 (s, 12H), 2.05 (s, 3H), 2.046 (s, 3H), 2.041 (s, 3H), 2.03 (s, 3H), 2.019 (s, 3H), 2.015 (s, 9H), 1.98 (s, 3H), 1.97 (s, 6H), 1.955 (s, 3H), 1.951 (s, 3H), 1.94 (s, 3H), 1.92 (s, 3H), 1.88 (s, 3H), 1.76-1.57 (m, 10H), 1.44-1.41 (m, 4H), 1.38-1.30 (m, 4H), 1.23-1.20 (m, 2H), 1.13-1.09 (m, 9H).

[0309] **Fully Deprotected Globo-H/Le^Y/Tn tripeptide 54.** 98%, white film; ^{1}H NMR (D_2O, 500 MHz) δ 5.30 (s, 1H, J=2.8 Hz), 5.25 (d, 1H, J=3.7 Hz), 5.13 (d, 1H, J=3.5 Hz), 4.91-4.87 (m, 3H), 4.75-4.74 (m, 1H), 4.63 (d, 1H, J=7.4 Hz), 4.57-4.48 (m, 3H), 4.41-4.38 (m, 2H), 4.31 (m, 1H), 4.29-4.24 (m, 6H), 4.18-4.12 (m, 3H), 4.05-3.56 (m, 58H), 3.50-3.46 (m, 3H), 3.32-3.24 (m, 5H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 6H), 1.82-1.65 (m, 14H), 1.47-1.42 (m, 6H), 1.29 (d, 3H, J=6.5 Hz), 1.25 (d, 3H, J=7.4 Hz), 1.23 (d, 3H, J=7.0 Hz); ^{13}C NMR (D_2O, 125 MHz) δ 175.11, 174.85, 174.81, 174.68, 174.44, 174.25, 174.13, 171.64, 164.59, 104.37, 103.71, 103.25, 102.78, 102.39, 102.44, 100.83, 100.59, 99.80, 99.67, 98.96, 97.34, 82.76, 79.18, 78.65, 77.55, 76.74, 76.50, 75.88, 75.78, 75.45, 75.24, 75.18, 75.00, 74.90, 73.96, 73.47, 73.35, 72.50, 72.32, 72.24, 72.09, 71.31, 71.24, 70.53, 70.44, 70.40, 70.16, 70.11, 69.90, 69.57, 69.50, 69.12, 68.93, 68.87, 68.66, 68.42, 68.22, 68.12, 68.08, 67.29, 67.17, 61.85, 61.68, 61.37, 61.34, 61.20, 60.74, 60.48, 60.19, 56.57, 54.02, 52.02, 50.41, 41.31, 37.09, 31.07, 30.79, 28.69, 28.64, 28.36, 22.72, 22.64, 22.40, 22.36, 22.25, 22.05, 22.01, 21.97, 21.93, 21.87, 21.84, 15.84, 15.70.

[0310] ***3) Preparation of Polycarbohydrate (globo H, Le^y, Tn) cluster-KLH conjugate using bifunctional cross linker method:***

[0311] Polycarbohydrate (globo H, Le^y, Tn) cluster is conjugated as described below using maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) which is a heterobifunctional reagant. At neutral pH it crosslinks amino groups with succinimide and then with thiol groups with

maleimide. The thiol group is provided by the cysteine residue of peptide backbone of cluster and the amino groups by the N-terminal and lysine side-chain of the KLH. After linkage MBS to KLH the unreacted MBS is purified by column and cross-linked to cysteine on the synthetic polycarbohydrate cluster. The unbound antigen is removed by passage of the reaction mixture through a CentriPrep 30 filter with a 30,000 molecular weight cut-off. The epitope ratio is then calculated by estimation of protein content by standard method and carbohydrate by high-pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) method.

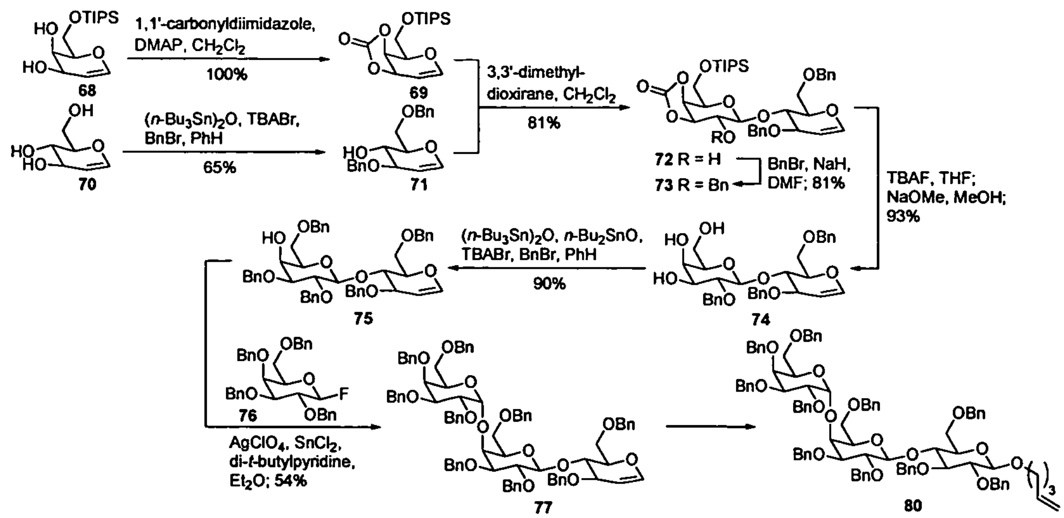
[0312] ***4) Preparation of Polycarbohydrate (TF, Le^y, Tn) cluster-KLH conjugate using bifunctional cross linker method:***

Polycarbohydrate (TF, Le^y, Tn) cluster is prepared in a similar fashion as that described in 3) above.

[0313] ***D. Example 4: Synthesis of n-alkylene Gb3 and conjugates thereof***

[0314] ***1) Discussion of Synthesis:***

[0315] As discussed above, in one aspect of the invention, the synthesis of Gb3 pentenyl glycoside **80** is provided (See Scheme 2). In one embodiment of the present invention, the synthesis of the disaccharide **73** starting from the known monosaccharides **68** and **70**, was first undertaken. Saccharide 68 was protected as its dioxolopyranone 69. Selective protection of **70** gave bibenzyl ether **71**. Coupling of **70** and **71** under suitable conditions gives the desired disaccharide **72**, which, upon reaction with BnBr in the presence of NaH, yields disccharide **73**. Partial deprotection of **73**, followed by reprotection the primary and equatorial hydroxyl groups as their benzyl ethers gives intermediate **75**, which, upon reaction with fluoro monosaccharide donor **76** under suitable conditions, gives trisaccharide construct **77**.



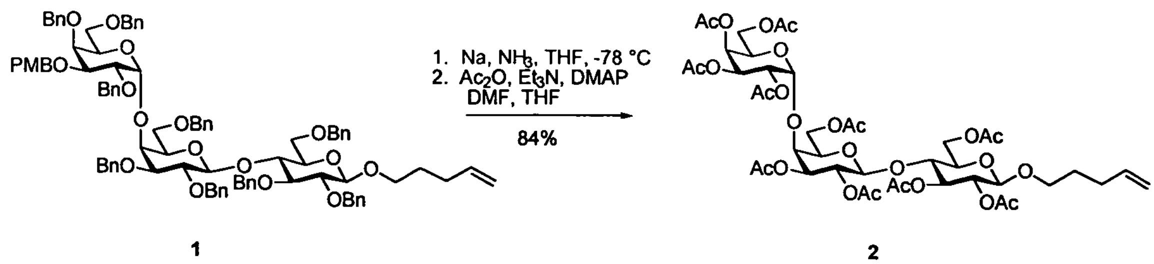
Scheme 2

[0316] In but one example, the use of a n-pentenyl glycoside was considered (For a review of n-pentenyl glycosides, see Fraser-Reid *et al.*, *Synlett*, **1992**, 927; Udodong *et al. J. Am. Chem. Soc.* **1993**, *115*, 7886; Merritt *et al. J. Am. Chem. Soc.* **1994**, *116*, 8334; Fraser-Reid *et al.* **1990**, *55*, 6068; Mootoo *et al. J. Am. Chem. Soc.* **1988**, *110*, 2662; Mootoo *et al. J. Am. Chem. Soc.* **1989**, *111*, 8540 and references therein). N-pentenyl glycosides are stable to a range of reaction conditions and reagents, but are readily activated for glycosidation reactions by treatment with a halogen oxidant. As a result of their stability and the neutral conditions required for their activation, pentenyl glycosides have been demonstrated to be valuable linkages for mechanistic and synthetic studies. Additionally, a terminal pentenyl group, or more generally a terminal alkenyl group, could also provide a handle for bioconjugation. Thus, in one embodiment, trisaccharide **77** may be reacted with pentenyl alcohol and anhydrous zinc chloride (Gordon *et al. Carbohydrate Res.* **1990**, *206*, 361) to afford the desired Gb3 n-pentenyl glycoside **80**. Indeed, with the pentenyl glycoside in place, global deprotection of **80** is possible.

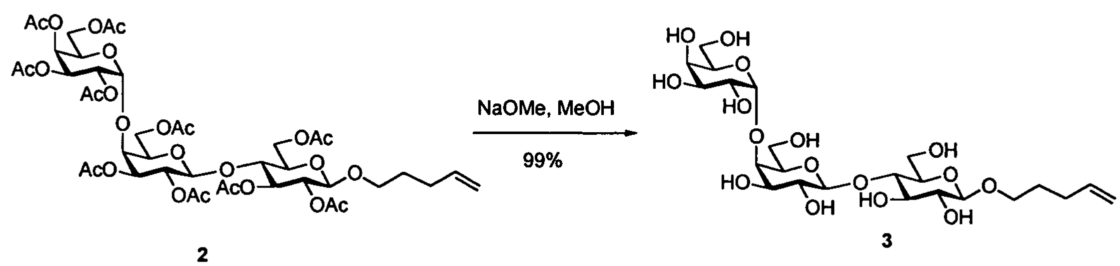
[0317] In certain embodiments, a Gb3 glycoconjugate may be prepared. Ozonolysis of intermediate **80**, thereby producing the corresponding aldehyde derivative, followed by coupling to KLH using reductive amination under the agency of sodium cyanoborohydride and global deprotection yields the desired Gb3-KLH glycoconjugate.

[0318] *2) Experimentals*

[0319] <u>N-pentenyl Gb3-KLH glycoconjugate</u>:



[0320] Sodium (38 mg, 1.69 mmol, 60.0 equiv) was added to condensed liquid ammonia (25 mL) at –78 °C. Pentenyl trisaccharide **1** (37 mg, 0.024 mmol; Ref: Allen, J.R.; Allen, J.G.; Zhang, X.F.; Williams, L.J.; Zatorski, A.; Ragupathi, G.; Livingston, P.O.; Danishefsky, S.J. *Chem. Eur. J.* **2000**, *6*, 1366-1375) in THF (0.5 mL) was added to the resulting blue solution, and the reaction was stirred at –78 °C for 45 min. MeOH (2 mL) was added to quench the reaction, followed by warming to rt to evaporate the condensed ammonia. Solid NH_4Cl (100 mg) was added, followed by filtration of the solids. The filtrate was concentrated, and the crude sugar was dissolved in 1:1:1 DMF:THF:Et_3N (1.5 mL), followed by DMAP (1 mg) and Ac_2O (0.14 mL, 1.48 mmol, 53.0 equiv). The reaction was stirred at rt for 15 h, diluted with EtOAc (5 mL) and washed with water (5 mL). The aqueous layer was extracted with EtOAc (2 × 5 mL). The combined organic layers were dried ($MgSO_4$), filtered, concentrated and purified by silica gel chromatography (72% EtOAc/hexanes) to afford peracetate **2** (20 mg, 84%) as a colorless oil.

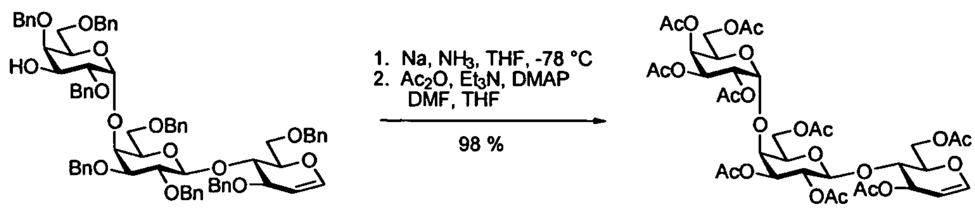


[0321] Peracetate **2** (18 mg, 0.018 mmol) in MeOH (1 mL) was treated with solid NaOMe (24 mg, 0.45 mmol, 25.0 equiv) and stirred at rt for 24 h. The reaction was quenched with Amberlite IR-120 acidic resin (20 mg), filtered, concentrated and purified by reverse phase chromatography (RP-18 silica gel, 20% MeOH/H_2O) to afford Gb3 trisaccharide **3** (11 mg, 99%) as a white solid: ^1H NMR (400 MHz, D_2O) δ 5.89 (ddt, J = 17.1, 10.4, 6.8 Hz, 1H), 5.07 (d, J = 17.4 Hz, 1H), 5.00 (d, J = 10.2 Hz, 1H), 4.92 (d, J = 3.7 Hz, 1H), 4.50 - 4.45 (m, 2H), 4.34 (t, J =

6.4 Hz, 1H), 4.01 (m, 2H), 3.96 (d, J = 11.0 Hz, 1H), 3.95 - 3.87 (m, 3H), 3.84 - 3.79 (m, 3H), 3.78 - 3.73 (m, 2H), 3.73 - 3.66 (m, 3H), 3.64 - 3.61 (m, 2H), 3.58 – 3.53 (m, 2H), 3.27 (t, J = 8.4 Hz, 1H), 2.15 – 2.03 (m, 2H), 1.74 – 1.67 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 140.0, 116.0, 104.5, 103.2, 101.5, 79.9, 78.6, 76.7, 76.0, 75.7, 74.2, 73.4, 72.1, 70.4, 70.1, 69.8, 61.7, 61.6, 61.3, 30.6, 29.2; LRMS (ESI) $M_{calc.}$ 572.2 for $C_{23}H_{40}O_{16}$, $(M+Na)_{found}$ 595.2.

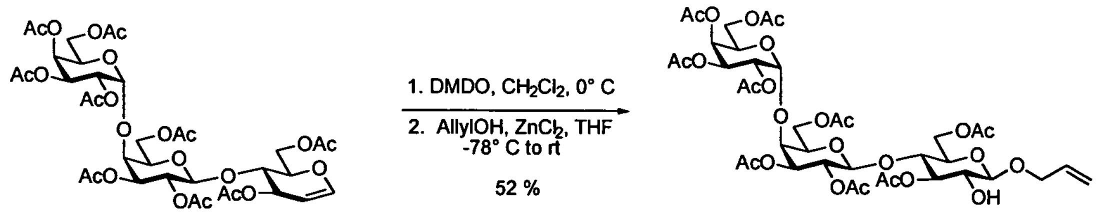
[0322] ***E. Example 5: Preparation of Gb3-glycoside-glycoamino acids and trimeric Gb3 glycopeptides 90 and 92***

[0323] *1) Synthesis of Gb3 glycoamino acid **10** (Figure 20B)*



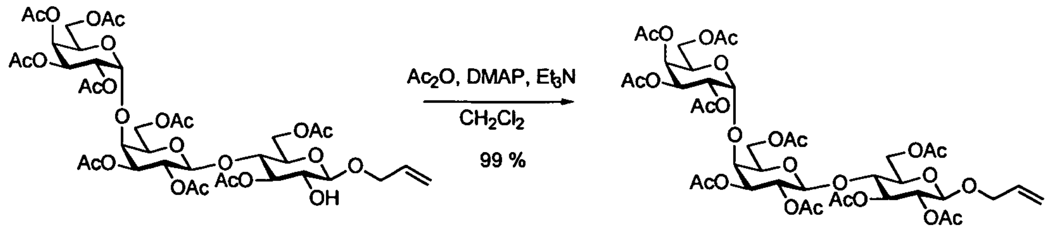
[0324] Sodium (193 mg, 8.41 mmol, 60.0 equiv) was added to condensed liquid ammonia (30 mL) at –78 °C. Glycal **4** (162 mg, 0.14 mmol; Ref: Park, T.K.; Kim, I.J.; Hu, S.; Bilodeau, M.T.; Randolph, J.T.; Kwon, O.; Danishefsky, S.J. *J. Am. Chem. Soc.* **1996**, *118*, 11488-11500) in THF (2.0 mL) was added to the resulting blue solution, and the reaction was stirred at –78 °C for 45 min. MeOH (5 mL) was added to quench the reaction, followed by warming to rt to evaporate the condensed ammonia. Solid NH_4Cl (445 mg, 8.41 mmol, 60.0 equiv) was added, followed by filtration of the solids. The filtrate was concentrated, and the crude sugar was dissolved in 1:1:1 DMF:THF:Et_3N (6.9 mL), followed by DMAP (10 mg) and Ac_2O (0.7 mL, 7.42 mmol, 53.0 equiv). The reaction was stirred at rt for 15 h, diluted with EtOAc (25 mL) and washed with water (25 mL). The aqueous layer was extracted with EtOAc (2 × 25 mL). The combined organic layers were dried ($MgSO_4$), filtered, concentrated and purified by silica gel chromatography (90% EtOAc/hexanes) to afford peracetate **5** (112 mg, 98%) as a white foam: ^1H NMR (400 MHz, $CDCl_3$) □ 6.36 (d, J = 6.0 Hz, 1H), 5.53 (d, J = 2.2 Hz, 1H), 5.38 – 5.32 (m, 2H), 5.15 – 5.10 (m, 2H), 4.95 (d, J = 3.3 Hz, 1H), 4.80 (dd, J = 5.9, 3.1 Hz, 1H), 4.74 (dd, J = 9.8, 2.1 Hz, 1H), 4.63 (d, J = 7.8 Hz, 1H), 4.47 (t, J = 6.8 Hz, 1H), 4.41 – 4.34 (m, 2H), 4.18 – 4.02 (m, 5H), 3.97 - 3.94 (m, 2H), 3.75 (t, J = 6.6 Hz, 1H), 2.08 (s, 3H),

2.06 (s, 6H), 2.03 – 2.01 (m, 12H), 1.99 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) □ 170.5, 170.4, 170.3, 170.3, 170.2, 169.9, 169.8, 169.6, 168.8, 145.2, 100.8, 99.3, 99.0, 77.2, 74.5, 74.0, 72.4, 71.5, 69.1, 68.5, 68.4, 67.6, 67.1, 66.9, 61.8, 61.2, 60.2, 20.9, 20.7, 20.6, 20.5, 20.4, 20.3; LRMS (ESI) $M_{calc.}$ 848.2 for $C_{36}H_{48}O_{23}$, $(M+Na)_{found}$ 871.3.



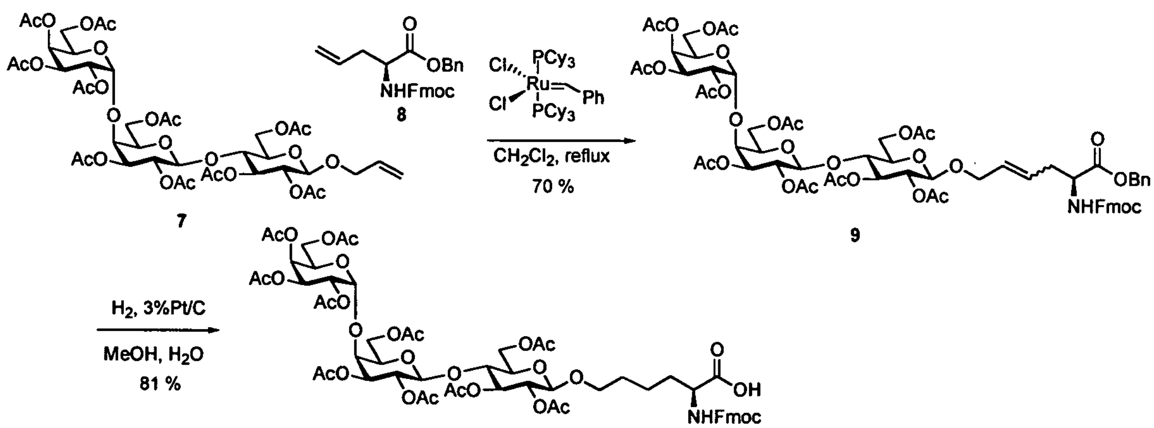
5 **6**

[0325] A solution of glycal **5** (181 mg, 0.213 mmol) in methylene chloride (1 mL) was cooled to 0 °C and treated with 2,2'-dimethyldioxirane (7 mL of a 0.06 M solution in acetone, 2.0 equiv). The reaction mixture was concentrated under vacuum after 45 min, and further dried by the addition of methylene chloride (1 mL) and subsequent evaporation, while maintaining the temperature at 0 °C. The crude glycal epoxide was dissolved in a 1:1 THF:allyl alcohol mixture (4 mL), cooled to –78 °C and was treated with $ZnCl_2$ (0.26 mL of a 1.0 M solution in diethyl ether, 1.2 equiv). The reaction was allowed to warm to rt slowly, and was maintained at that temperature for 15 h. EtOAc (20 mL) was added, and the solution was washed with a saturated solution of $NaHCO_3$ (20 mL), brine (20 mL), dried ($MgSO_4$), filtered, concentrated and purified by silica gel chromatography (60 - 76% EtOAc/hexanes) to afford allyl glycoside **6** (99 mg, 52%) as a colorless oil, along with 64 mg (34%) of the corresponding □-manno diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$) □ 5.89 (ddt, J = 16.8, 11.4, 6.2 Hz, 1H), 5.55 (d, J = 2.2 Hz, 1H), 5.34 (dd, J = 11.0, 3.2 Hz, 1H), 5.29 (d, J = 17.2 Hz, 1H), 5.20 (d, J = 10.5 Hz, 1H), 5.15 (dd, J = 11.0, 3.4 Hz, 1H), 5.10 – 5.05 (m, 2H), 4.96 (d, J = 3.4 Hz, 1H), 4.71 (dd, J = 10.8, 2.2 Hz, 1H), 4.56 – 4.30 (m, 6H), 4.16 – 4.05 (m, 5H), 3.99 (s, 1H), 3.82 - 3.68 (m, 2H), 3.61 – 3.58 (m, 2H), 3.47 (t, J = 8.5 Hz, 1H), 2.72 (bs, 1H), 2.19 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.05 – 2.02 (m, 15H), 1.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) □ 170.6, 170.5, 170.4, 170.3, 169.9, 169.4, 168.7, 133.3, 118.2, 101.3, 100.8, 99.5, 76.8, 76.2, 74.9, 74.7, 72.7, 72.6, 72.5, 71.7, 70.3, 68.8, 68.7, 67.8, 67.0, 66.9, 62.3, 61.3, 60.2, 20.8, 20.7, 20.6, 20.6, 20.5, 20.5, 20.4; LRMS (ESI) $M_{calc.}$ 922.3 for $C_{39}H_{54}O_{25}$, $(M+H)_{found}$ 923.3, $(M+Na)_{found}$ 945.3.



6 7

[0326] A solution of alcohol **6** (80 mg, 0.086 mmol) in methylene chloride (2.5 mL) was treated with Et_3N (0.06 mL, 0.43 mmol, 5.0 equiv), DMAP (10 mg, 0.086 mmol, 1.0 equiv) and acetic anhydride (0.035 mL, 0.346 mmol, 4.0 equiv) and stirred at rt. After 5 h, the reaction mixture was poured into a saturated solution of $NaHCO_3$ (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried ($MgSO_4$), filtered, concentrated and purified by silica gel chromatography (60 - 68% EtOAc/hexanes) to afford peracetate **7** (81 mg, 99%) as a colorless foam: ^{1}H NMR (400 MHz, $CDCl_3$) □ 5.82 (ddt, J = 16.8, 10.7, 5.8 Hz, 1H), 5.57 (d, J = 2.3 Hz, 1H), 5.37 (dd, J = 11.0, 3.2 Hz, 1H), 5.27 (d, J = 2.2 Hz, 1H), 5.22 – 5.14 (m, 3H), 5.08 (dd, J = 10.8, 7.8 Hz, 1H), 4.96 (d, J = 3.5 Hz, 1H), 4.91 (t, J = 8.6 Hz, 1H), 4.71 (dd, J = 10.8, 2.2 Hz, 1H), 4.58 – 4.39 (m, 5H), 4.29 (dd, J = 13.2, 4.8 Hz, 1H), 4.17 – 4.04 (m, 5H), 3.95 (s, 1H), 3.81 - 3.73 (m, 2H), 3.63 – 3.59 (m, 1H), 2.11 (s, 3H), 2.10 (s, 3H), 2.06 (s, 6H), 2.05 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 6H), 1.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) □ 170.6, 170.4, 170.0, 169.6, 169.6, 169.4, 168.8, 133.3, 117.5, 101.0, 99.5, 99.2, 76.8, 76.4, 73.0, 72.7, 72.4, 71.7, 71.6, 69.9, 68.9, 68.7, 67.8, 67.0, 67.0, 62.1, 61.2, 60.2, 20.8, 20.8, 20.6, 20.6, 20.5, 20.5, 20.4; LRMS (ESI) $M_{calc.}$ 964.3 for $C_{41}H_{56}O_{26}$, $(M+H)_{found}$ 965.2, $(M+Na)_{found}$ 987.3.



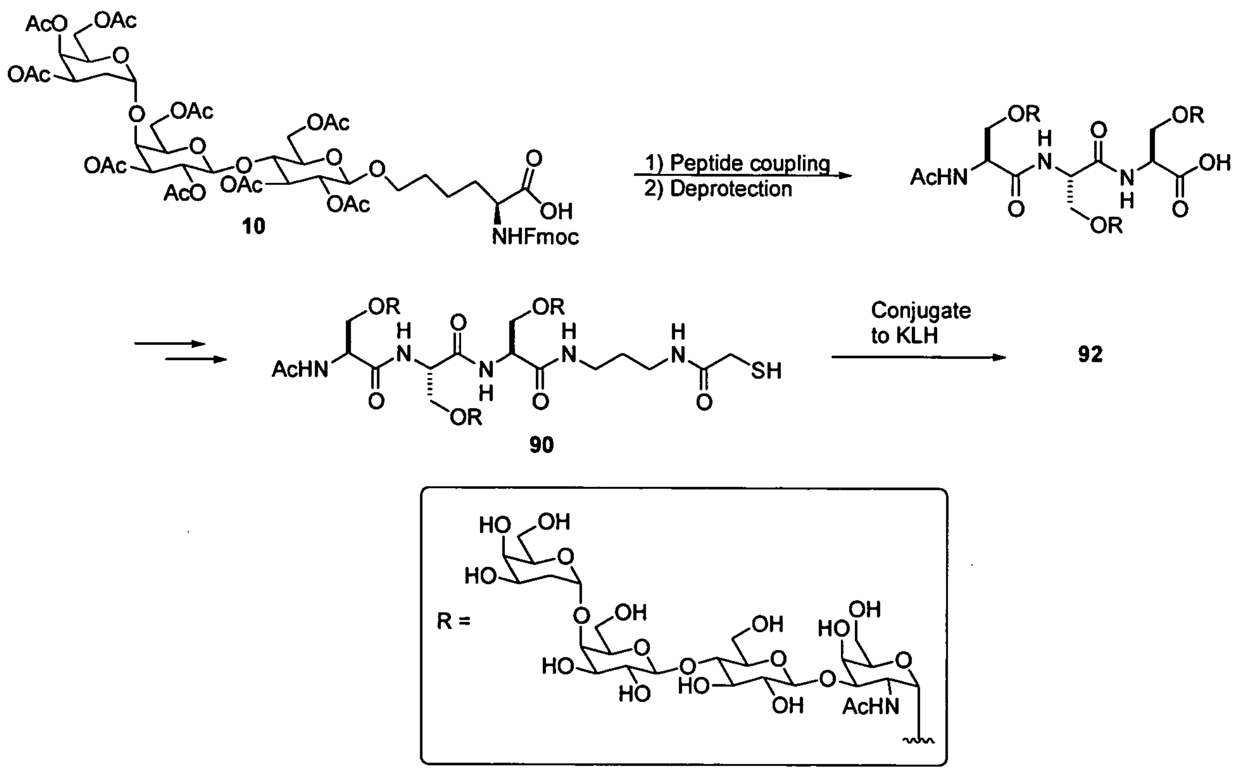
10

[0327] Peracetylated Gb3 allyl glycoside **7** (10 mg, 0.01 mmol) and Fmoc-L-allylglycine benzyl ester **8** (25 mg, 0.05 mmol, 5.0 equiv) in methylene chloride (0.05 mL) were treated

bis(tricyclohexylphosphine) ruthenium Grubbs catalyst (1 mg, 0.001 mmol, 0.1 equiv). The reaction was heated to reflux. After 6h, another 0.1 equiv of catalyst was further added. The reaction was cooled to rt after 12 h (total) and purified by silica gel chromatography (40% acetone/hexanes) to afford metathesis product **9** (9 mg, 70 %). The *E*:*Z* mixture **9** (78 mg, 0.057 mmol) was dissolved in 0.55 mL methanol:water (10:1) and treated with 3% Pt/C (70 mg). The reaction flask was stirred under a hydrogen balloon for 12 h, followed by filtration to remove the solid catalyst. The filtrate was concentrated and purified by silica gel chromatography (EtOAc - 1% AcOH/EtOAc) to afford Gb3 amino acid **10** (58 mg, 81%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 2H), 7.60 (d, J = 7.5 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.31 (t, J = 7.5 Hz, 2H), 5.59 (d, J = 2.6 Hz, 1H), 5.50 (d, J = 7.9 Hz, 1H), 5.39 (dd, J = 11.0, 3.0 Hz, 1H), 5.22 - 5.16 (m, 2H), 5.09 (dd, J = 10.4, 8.0 Hz, 1H), 5.01 (s, 1H), 4.98 (d, J = 3.2 Hz, 1H), 4.87 (t, J = 8.5 Hz, 1H), 4.75 – 4.71 (m, 1H), 4.53 - 4.38 (m, 6H), 4.23 (t, J = 6.9 Hz, 1H), 4.19 - 4.08 (m, 4H), 4.00 (s, 1H), 3.81 - 3.73 (m, 3H), 3.64 – 3.61 (m, 1H), 3.48 – 3.46 (m, 1H), 2.13 (s, 3H), 2.10 (s, 3H), 2.06 (s, 9H), 2.05 (s, 6H), 2.03 (s, 3H), 2.02 (s, 3H), 1.98 (s, 3H), 1.92 - 1.89 (m, 1H), 1.83 – 1.81 (m, 1H), 1.60 - 1.54 (m, 2H), 1.30 - 1.24 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) □ 175.1, 170.7, 170.6, 170.5, 170.4, 170.0, 169.9, 169.6, 169.5, 168.8, 156.0, 143.7, 141.2, 134.4, 129.7, 128.9, 128.5, 127.7, 127.0, 125.0, 119.9, 101.0, 100.4, 99.5, 79.1, 77.2, 76.3, 73.0, 72.7, 72.5, 71.8, 69.3, 68.9, 68.8, 67.8, 67.0, 62.1, 60.2, 53.6, 47.1, 31.6, 28.8, 21.5, 20.9, 20.8, 20.6, 20.6, 20.5, 20.4; LRMS (ESI) $M_{calc.}$ 1275.4 for $C_{59}H_{73}NO_{30}$, $(M+H)_{found}$ 1276.3, $(M+Na)_{found}$ 1298.4.

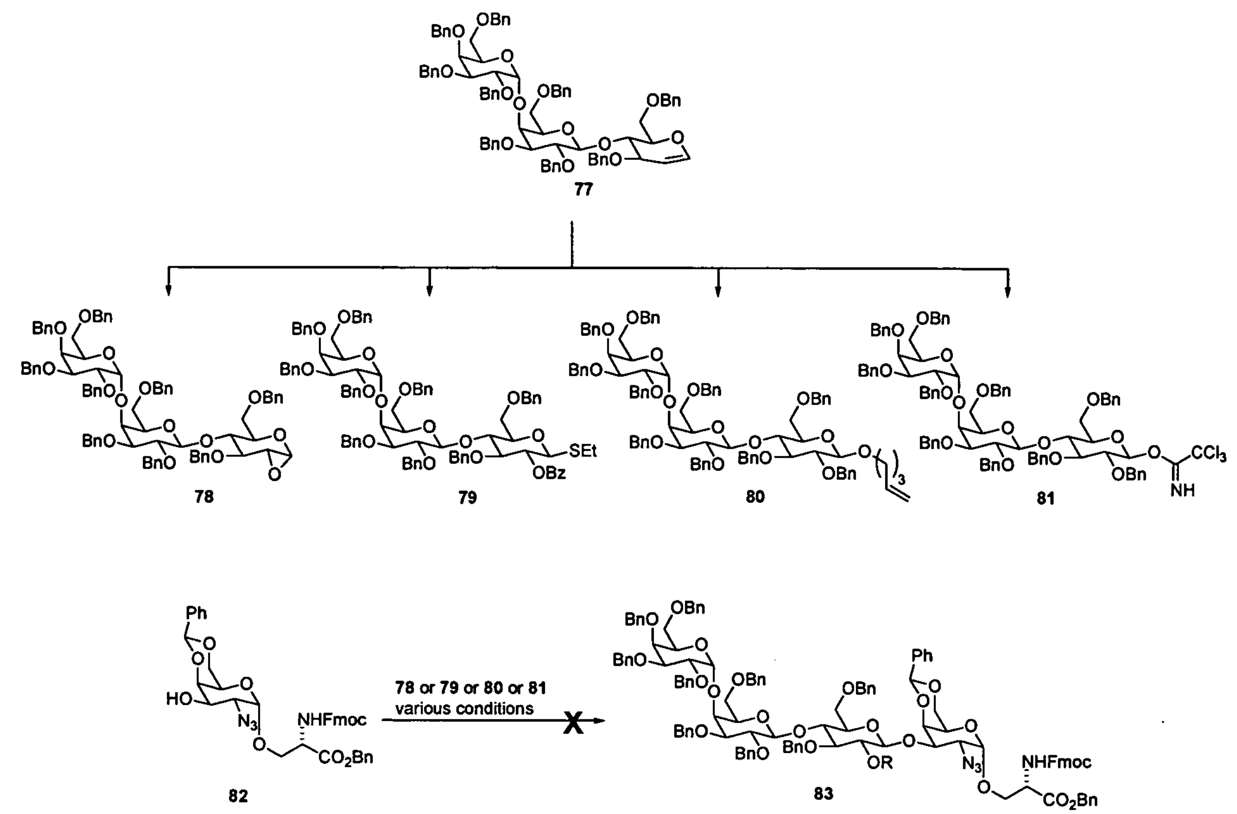
[0328] *2) Synthesis of trimeric Gb3 glycopeptides **90** and **92***

[0329] As depicted below, peptide coupling of intermediate **10**, followed by attachement of the resulting trimeric peptide to a suitable linker and global deprotection yields the desired trimeric glycopeptide **90**. Conjugation to KLH under suitable conditions affords trimeric glycopeptide **92**.



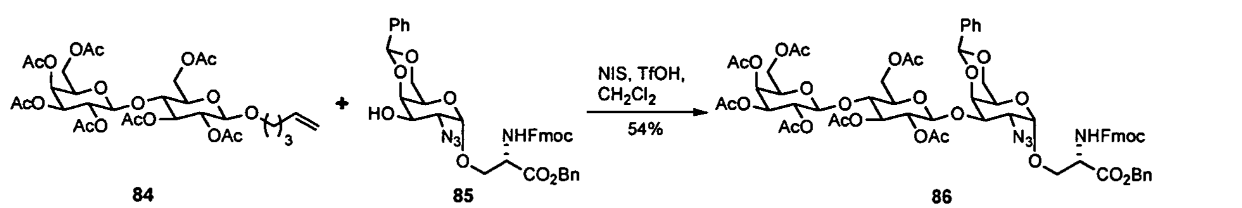
[0330] **2) Synthesis of trimeric Gb3 glycopeptides 91 and 93 (Figure 21) - Discussion of Synthetic Methods:**

[0331] As described above, Gb3 glycoamino acids may be prepared. In certain embodiments, Gb3 glycoamino acid constructs are provided comprising a Gb3 epitope linked to an aliphatic amino acid moiety through a monosaccharide moiety. The synthetic route that we have been investigating towards this end relies on the initial synthesis of a suitably protected Gb3 glycal (77), which was carried out as detailed herein (Figures 20A and B). With the glycal in hand, we began an investigation into incorporating monosaccharide amino acid **82** acceptor at the reducing end. Despite the use of several different donor types (**78, 79, 80, 81**), efforts directed at coupling the Gb3 trisaccharide and the acceptor **82** were unsuccessful (Scheme 2).



Scheme 2

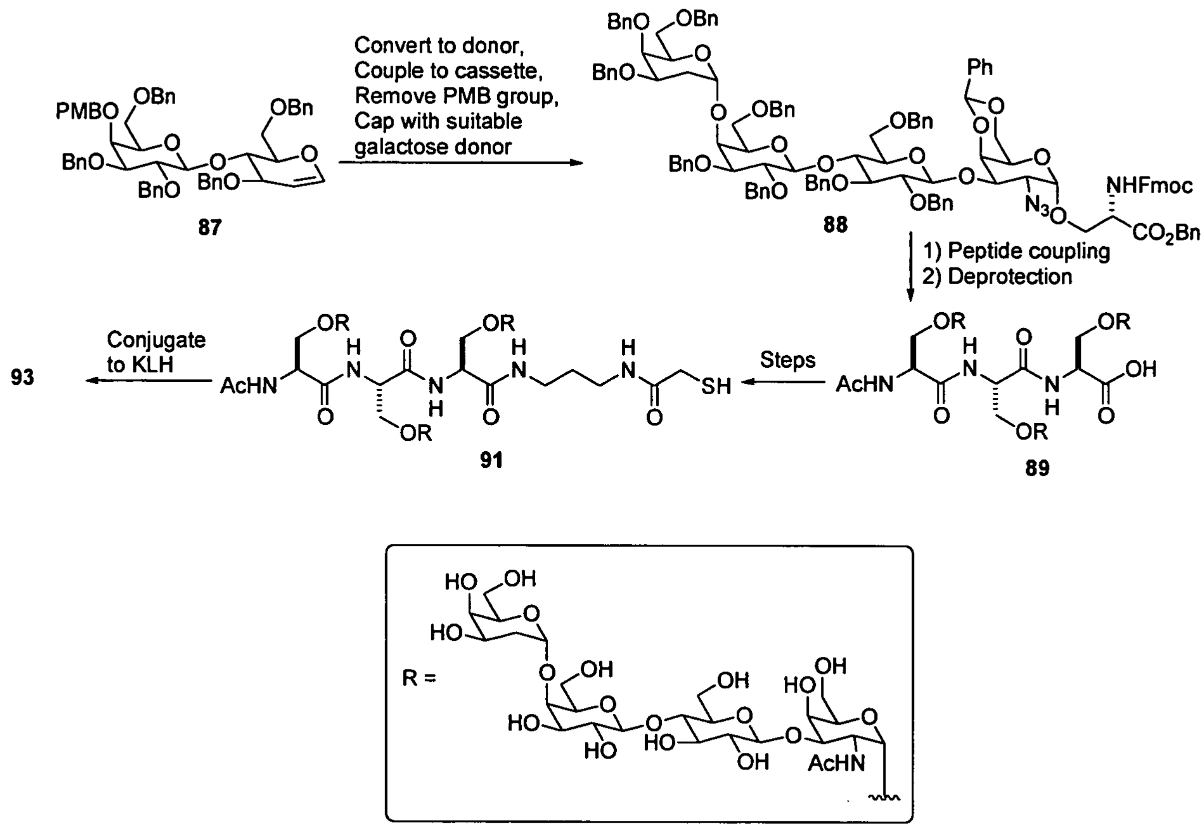
[0332] We have, thus, been led to investigate an alternative approach, in which monosaccharide amino acid acceptor **82** is coupled to the AB disaccharide portion of Gb3, and then the resulting adduct is coupled with the C residue, thereby generating the required Gb3-GalNAc glycoamino acid. Initial experiments in this vein have shown that the coupling is, indeed, possible using the peracylated lactose donor **84** (Scheme 3).



Scheme 3

[0333] Consequently, the orthogonally protected lactal residue **87** has been prepared (Scheme 4) for introduction of monosaccharide amino acid acceptor **82** into this piece. As depicted in Scheme 4 (and in more details in Figure 21), conversion of **87** to the corresponding donor, followed by coupling with monosaccharide amino acid acceptor **82**, removal of the PMB

group and capping with a suitable galactose donor generates the desired glycoamino acid **88**. Hence, in certain embodiments, clustered variants of Gb3 are provided. Given the structural similarity of Gb3 to the reducing end of Globo-H, the approach developed to synthesize the Gb3-GalNAc glycoamino acid will potentially be applicable to the synthesis of the corresponding glycoamino acid derived from Globo-H.



Scheme 4

[0334] ***F. Example 6: Preparation of clusters of clusters***

[0335] As discussed herein, certain carbohydrate epitopes are often significantly overexpressed on the surface of transformed cells.[1] The isolation and identification of these differential antigens has allowed for their use in the development of anti-cancer vaccines.[2] These vaccines, normally consisting of the carbohydrate antigen attached through a linker domain to an immunogenic carrier protein, have been investigated for their ability to generate an immune response directed at eliminating circulating tumor cells and micrometastases.[3] One of the limiting factors in exploring the utility of these cell-free glycoconjugate vaccines is the limited availability of purified tumor-associated carbohydrate antigens. Many laboratories have

increasingly come to rely on total chemical synthesis for producing probe structures to be evaluated in such constructs.[4] We have become progressively active in developing multiple synthetic methods for the syntheses of these antigens as well as investigating the ideal technique for creating vaccine constructs.

[0336] Based on our earlier investigations, we have come to prefer the display of tumor antigens on a peptide backbone. The desired *N*-protected glycoamino acids are individually synthesized and then the vaccine assembled by iterative peptide couplings. The strategy in our laboratory for introducing amino acid functionality to tumor-associated carbohydrate antigens first involved glycosylations utilizing natural amino acids (Ser, Thr) to provide core mucin structures.[5] More recently, our efforts in the total synthesis of carbohydrate-based anti-tumor vaccines have led us to favor non-natural amino acids as components of our peptide-linked vaccines.[6] In principle, such unnatural linkages might result in an enhanced immune response. Additionally, the use of non-natural amino acids, in particular those containing long, aliphatic side chains that create greater distance between the peptide backbone and the glycosides, may be critical to the success of the glycopeptide synthesis. One vaccine employing these non-natural amino acids as linkers to carbohydrate domains has undergone preliminary investigation and demonstrated proof of principle in its effectiveness at inducing immunogenicity in murine hosts.[7]

[0337] We have come to depend on three different protocols for facilitating the introduction of the non-natural amino acid moiety to the carbohydrate domain. Specifically, transformation of *O*-pentenyl glycosides *via* an ozonolysis-Wittig-asymmetric hydrogenation sequence,[6] cross-metathesis of *O*-allyl glycosides with allylglycine[8] and the most recently disclosed direct glycosylation of hydroxynorleucine with a suitable glycosyl donor[9] all result in the same type side chain, multiple methylene units, between the α-carbon of the amino acid and the carbohydrate antigen.

[0338] Our initial vaccine constructs were primarily monomeric in composition, consisting of a single glycolipid tumor-associated carbohydrate antigen attached to KLH. Later, we examined synthetic mucin-based antigen conjugates that contained several copies of the particular antigen displayed in a single construct and we refer to these types of glycopeptides as clustered conjugate vaccines. In the monomeric vaccines both the clustered and non-clustered, the generated immune response was only directed toward one antigen and could only be effective

if all the targeted transformed cells expressed that antigen.[3a] Since cancerous cells are heterogeneous with regard to the types of antigens displayed on their cell surfaces along with the fact that these antigens may be displayed differentially during the processes of cell development,[10] the current vaccines, those containing a single antigen, may neglect a significant population of transformed cells.

[0339] Our vision for a new type of vaccine construct was based upon the concept of multiantigenicity. In principle, there are two vaccination strategies for targeting a range of tumor-associated antigens leading to a diverse immune response in which the likelihood of immunoevasion by the heterologous cell types is reduced. The first strategy involves immunization using a cocktail mixture of the monomeric conjugate vaccines.[11] A patient is given a mixture of 5-7 different monomeric KLH-vaccines that is representative of the most commonly occurring antigens for their specific type of cancer. This approach, which has been termed *polyvalent*, has potential limitations. From a regulatory perspective, difficulties could be encountered in obtaining approval for each of the components involved. However, from an immunological viewpoint, there lies the potential for carrier-induced immune suppression as well as a decrease in antibody production against the targeted antigens.[7] These complications could perhaps be avoided through combination of the desired antigens on the same conjugate vaccine. In this case, and the one which we prefer, involves the placement of at least two different antigens on a peptide backbone followed by conjugation to carrier resulting in a *unimolecular multiantigenic* vaccine. We have demonstrated proof-of-principle of this methodology. Our initial vaccines,[7] which contained three different antigens, were able to elicit antibodies in murine hosts that not only recognized the individual antigens of interest, but also were able to bind to cancer cells that specifically expressed those same antigens. We have since synthesized a unimolecular multiantigenic vaccine that is comprised of five different antigens. The five antigens included (Tn, TF, STn, Lewis^y, and Globo-H) are the most commonly occurring carbohydrate antigens found in cancer of the prostate as well as being indicated in breast and ovarian cancers.

[0340] Having demonstrated the viability of mounting a multifaceted immune response utilizing the unimolecular multiantigenic approach, our attention has been turned to optimizing this approach. Specifically, the next generation of vaccines currently being pursued combine the concepts of multiantigenicity and clustering resulting in the type of compound illustrated below:

clusters of clusters. Examples of such clustered constructs, and exemplary synthetic approaches for making them, are depicted in Figures 26-34. Additional synthetic guidance is provided in the "Experimental" section below. The display of the carbohydrates shown here may be a more accurate mimic of that found on the surface of transformed cells.[12] The antigens of the original mucin-type (Tn, TF, STn, etc.) are typically found as constituents of glycopeptides in which there are multiple, repeating serine and threonine residues that are glycosylated resulting in a clustered effect. This clustering effect is not limited, however, to this group of carbohydrate antigens. The glycosphingolipids (Lewis^y, Globo-H, KH1, fucosylGM_1, GB_3, etc.), described as such by their ceramide domains, are known to cluster together in the cell membrane thus providing glycospingolipid (GSL) domains or patches that display the carbohydrate antigens. The cluster of clusters vaccine may more accurately represent the architecture of the carbohydrate antigens on the surfaces of transformed cells; and, in as much, may be better suited for a robust immune response against a higher population of those cells.

[0341] 1. (a) Hakomori, S. *Adv. Cancer Res.* **1989**, *52*, 257; (b) Kjeldsen, T. B.; Clausen, H.; Hirohashi, S.; Ogawa, T.; Iijima, H.; Hakomori, S. *Cancer Res.* **1988**, *48*, 2214; (c) Springer, G. F. *Science* **1984**, *224*, 1198.

[0342] 2. (a) Fung, P. Y. S.; Madej, M.; Koganty, R. R.; Longenecker, B. M. *Cancer Res.* **1990**, *50*, 4308; (b) Livingston, P. O.; Ritter, G.; Srivastava, P.; Padavan, M.; Calves, M. J.; Oettgen, H. F.; Old, L. J. *Cancer Res.* **1989**, *49*, 7045.

[0343] 3. (a) Danishefsky, S. J.; Allen, J. R. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 836; (b) Livingston, P. O.; Zhang, S. L.; Lloyd, K. O. *Cancer Immunol. Immunother.* **1997**, *45*, 1; (c) Livingston, P. O.; Ragupathi, G. *Cancer Immunol. Immunother.* **1997**, *45*, 10.

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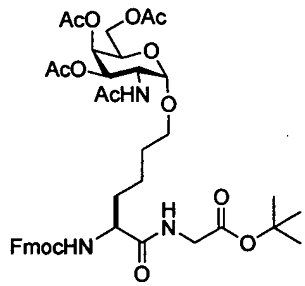
[0353] ***Experimental***

[0354] General Procedure A for peptide couplings: To a solution of carboxylic acid (1.05 eq) in CH_2Cl_2:DMF (1:1 mixture at a final concentration of 0.05 to 0.01 M) at -10 °C was added triethylamine [TEA] (1.05 eq), hydroxybenzotriazole [HOBt] (1.5 eq), and diethylaminopropyl carbodiimide [EDCI] (1.25 eq). The mixture was stirred for 30 minutes and then the amine (1.0 eq) was added. The reaction was stirred at –10 °C for 1 hour and then the cooling bath allowed to expire during the next 12 hours. The reaction was concentrated in vacuo and purified on silica gel via flash column chromatography (gradient of MeOH in CH_2Cl_2).

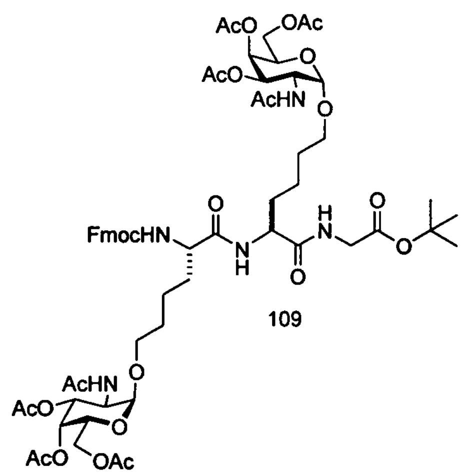
[0355] General Procedure B for Fmoc deprotections: The Fmoc protected compound was dissolved in a 1:1 mixture of DMF:morpholine (final concentration of 0.005 M) and stirred

at room temperature for 2 hours. The solvents were removed in vacuo and the residue purified on silica gel via flash column chromatography (gradient of MeOH in CH_2Cl_2).



108

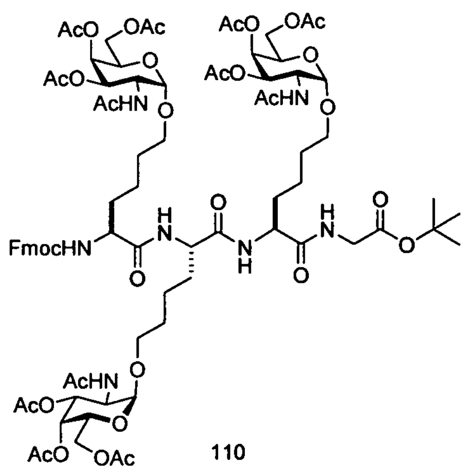
[0356] Fmoc-Nle(α-Tn)-Gly-OtBu **108** (Figure 26). Acid **107** (40 mg, 57.2 μmol) was reacted with Gly-OtBu according to General Procedure A. Purification (0 to 4% MeOH in CH_2Cl_2) provided the product as a white solid (44 mg, 54.2 μmol) in 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, 2H, J = 7.5 Hz), 7.59-7.61 (m, 2H), 7.39 (t, 2H, J = 7.4 Hz), 7.30 (t, 2H, J = 7.5 Hz), 6.76 (bs, 1H), 6.24 (d, 1H, J = 9.5 Hz), 5.78 (d, 1H, J = 8.0 Hz), 5.39 (d, 1H, J = 2.8 Hz), 5.20-5.22 (m, 1H), 4.86 (d, 1H, J = 3.5 Hz), 4.56-4.62 (m, 1H), 4.41 (m, 2H), 4.04-4.26 (m, 5H), 3.85-3.91 (m, 2H), 3.72 (m, 1H), 3.45 (m, 1H), 2.16 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.40-1.80 (m, 6H), 1.45 (s, 9H); MS (LR-ESI) calculated for $C_{41}H_{53}N_3O_{14}Na$ $[M+Na]^+$ 834.4, found 834.3.



109

[0357] Fmoc-Nle(α-Tn)-Nle(α-Tn)-Gly-OtBu **109** (Figure 26). Dipeptide **108** (44 mg, 54.2 μmol) was deprotected according to General Procedure B. Purification (5 to 20% MeOH in CH_2Cl_2) provided the product as a white solid (28 mg, 47.5 μmol) in 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.34 (d, 1H, J = 9.5 Hz), 5.38 (d, 1H, J = 2.7 Hz), 5.15 (dd, 1H, J = 11.4, 3.2

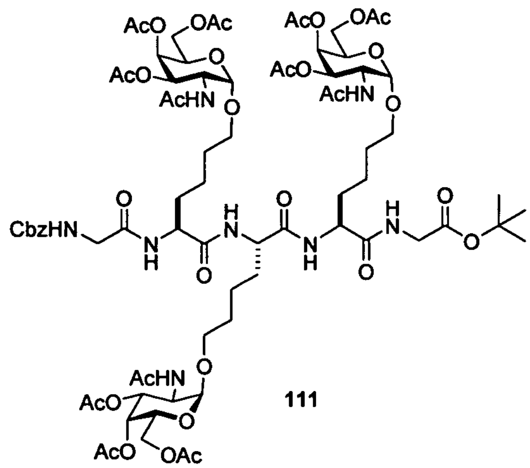
Hz), 4.87 (d, 1H, J = 3.6 Hz), 4.55-4.61 (m, 1H), 4.05-4.18 (m, 3H), 3.84-3.99 (m, 2H), 3.72 (dt, 1H, J = 9.7, 6.0 Hz), 3.43-3.48 (m, 1H), 2.16 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.40-1.80 (m, 6H), 1.48 (s, 9H). The free amine (28 mg, 47.5 μmol) was reacted with acid **107** (35 mg, 49.9 μmol) according to General Procedure A. Purification (0 to 6% MeOH in CH_2Cl_2) provided the product as a white solid (48.4 mg, 38.1 μmol) in 80% yield. ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, 2H, J = 7.5 Hz), 7.61 (d, 2H, J = 7.3 Hz), 7.40 (t, 2H, J = 7.4 Hz), 7.27-7.33 (m, 2H), 6.94 (bs, 1H), 6.84 (d, 1H, J = 7.7 Hz), 6.30 (d, 1H, J = 9.6 Hz), 6.25 (d, 1H, J = 9.6 Hz), 5.98 (d, 1H, J = 8.0 Hz), 5.18-5.50 (m, 4H), 4.86 (d, 1H, J = 3.4 Hz), 4.81 (d, 1H, J = 2.9 Hz), 4.48-4.62 (m, 5H), 3.38-4.24 (m, 13H), 2.17 (s, 3H), 2.15 (s, 3H), 2.04 (s, 6H), 2.03 (s, 3H), 1.99 (s, 6H), 1.98 (s, 3H), 1.30-1.90 (m, 12H), 1.44 (s, 9H); MS (LR-ESI) calculated for $C_{61}H_{83}N_5O_{24}Na$ $[M+Na]^+$ 1292.5, found 1292.4.



110

[0358] Fmoc-Nle(α-Tn)-Nle(α-Tn)-Nle(α-Tn)-Gly-OtBu **110** (Figure 26). Tripeptide **109** (48.4 mg, 38.1 μmol) was deprotected according to General Procedure B. Purification (5 to 20% MeOH in CH_2Cl_2) provided the product as a white solid (41.2 mg, 38.1 μmol) in quantitative yield. ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, 1H, J = 8.2 Hz), 6.90 (t, 1H, J = 5.1 Hz), 6.61 (d, 1H, J = 9.5 Hz), 6.31 (d, 1H, J = 9.5 Hz), 5.37 (d, 2H, J = 3.5 Hz), 5.14-5.21 (m, 2H), 4.87 (m, 2H), 4.59 (m, 2H), 4.45 (m, 1H), 4.08-4.20 (m, 6H), 3.89 (d, 2H, J = 5.1 Hz), 3.72-3.75 (m, 2H), 3.43-3.48 (m, 2H), 2.16 (s, 3H), 2.15 (s, 3H), 2.05 (s, 6H), 2.00 (s, 3H), 1.99 (s, 6H), 1.98 (s, 3H), 1.30-1.90 (m, 12H), 1.45 (s, 9H). The free amine (41.2 mg, 39.3 μmol) was reacted with acid **107** (29 mg, 41.2 μmol) according to General Procedure A. Purification (2 to 8% MeOH in CH_2Cl_2) provided the product as a white solid (52.8 mg, 30.5 μmol) in 78% yield.

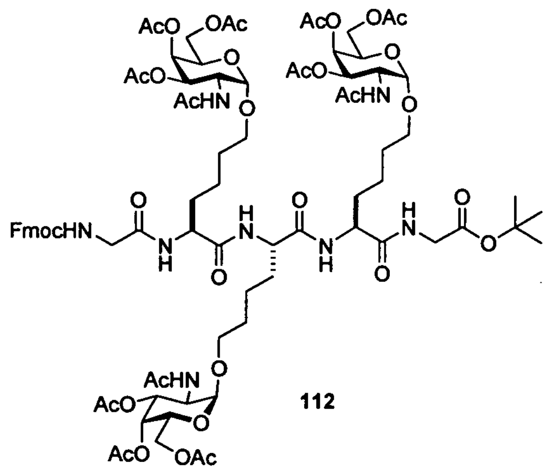
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, 2H, J = 7.5 Hz), 7.60 (d, 2H, J = 7.0 Hz), 7.40 (t, 2H, J = 7.4 Hz), 7.28-7.31 (m, 2H), 6.99 (d, 1H, J = 7.9 Hz), 6.94 (d, 1H, J = 9.0 Hz), 6.62 (m, 2H), 6.26 (m, 1H), 6.05 (d, 1H, J = 9.4 Hz), 5.41 (d, 1H, J = 3.0 Hz), 5.39 (d, 1H, J = 2.9 Hz), 5.36 (d, 1H, J = 2.6 Hz), 5.08-5.30 (m, 3H), 4.93 (d, 1H, J = 3.7 Hz), 4.87 (d, 1H, J = 3.4 Hz), 4.83 (d, 1H, J = 3.3 Hz), 3.37-4.61 (m, 24H), 2.17 (s, 3H), 2.16 (s, 3H), 2.15 (s, 3H), 2.06 (s, 3H), 2.05 (s, 6H), 2.04 (s, 3H), 2.03 (s, 3H), 1.99 (s, 6H), 1.98 (s, 3H), 1.96 (s, 3H), 1.25-1.95 (m, 18H), 1.45 (s, 9H); MS (LR-ESI) calculated for $C_{81}H_{113}N_7O_{34}Na$ $[M+Na]^+$ 1750.7, found 1750.8.



111

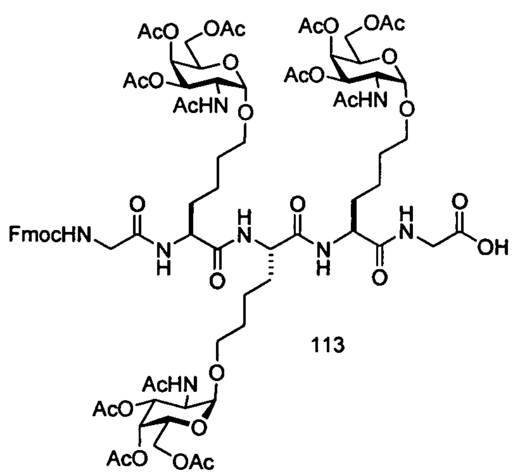
[0359] Cbz-Gly-Nle(α-Tn)-Nle(α-Tn)-Nle(α-Tn)-Gly-OtBu **111** (Figure 26). Tetrapeptide **110** (52.3 mg, 30.5 μmol) was deprotected according to General Procedure B. Purification (5 to 20% MeOH in CH_2Cl_2) provided the product as a white solid (42.2 mg, 28.0 μmol) in 92% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, 1H, J = 7.9 Hz), 6.96-7.01 (m, 2H), 6.64 (d, 1H, J = 9.4 Hz), 6.44 (d, 1H, J = 9.4 Hz), 6.25 (d, 1H, J = 9.5 Hz), 5.38 (m, 3H), 5.17 (m, 3H), 4.87 (m, 3H), 4.35-4.60 (m, 5H), 4.09-4.19 (m, 10H), 3.90 (m, 2H), 3.70 (m, 3H), 3.45 (m, 3H), 2.16 (s, 9H), 2.05 (s, 9H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 9H), 1.98 (s, 3H), 1.25-1.90 (m, 18H), 1.46 (s, 9H); MS (LR-ESI) calculated for $C_{66}H_{103}N_7O_{32}Na$ $[M+Na]^+$ 1528.7, found 1528.8. The free amine (15.8 mg, 10.5 μmol) was reacted with Cbz-Gly-OH (2.3 mg, 11.0 μmol) according to General Procedure A. Purification (2 to 8% MeOH in CH_2Cl_2) provided the product as a white solid (14.3 mg, 8.42 μmol) in 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27-7.37 (m, 5H), 7.19 (m, 1H), 7.08 (d, 1H, J = 7.8 Hz), 6.90 (m, 1H), 6.75 (m, 1H), 6.56 (m, 1H), 6.32 (d, 1H, J = 9.5 Hz), 5.39 (m, 3H), 5.09-5.18 (m, 5H), 4.86-4.92 (m, 3H), 4.32-4.62 (m, 6H), 4.06-4.19 (m, 9H), 3.85-3.92 (m, 4H), 3.67 (m, 3H), 3.43 (m, 3H), 2.17 (s, 3H), 2.16 (s,

6H), 2.06 (s, 3H), 2.04 (s, 6H), 2.02 (s, 3H), 2.00 (s, 6H), 1.97 (s, 3H), 1.96 (s, 3H), 1.90 (s, 3H), 1.25-1.95 (m, 18H), 1.45 (s, 9H); MS (LR-ESI) calculated for $C_{76}H_{112}N_8O_{35}Na$ $[M+Na]^+$ 1719.7, found 1719.8.



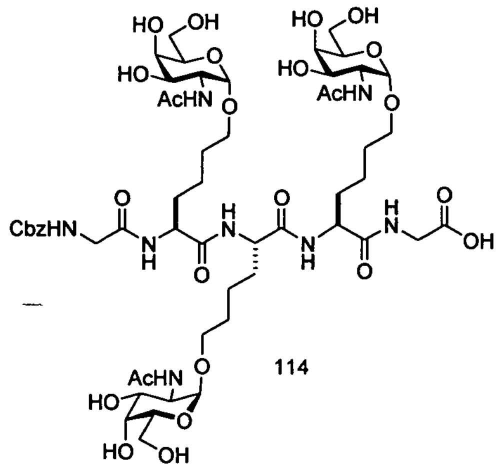
112

[0360] Fmoc-Gly-Nle(α-Tn)-Nle(α-Tn)-Nle(α-Tn)-Gly-OtBu **112** (Figure 26). Tetrapeptide amine from above (42.2 mg, 28.0 μmol) was reacted with Fmoc-Gly-OH according to General Procedure A. Purification (2 to 9% MeOH in CH_2Cl_2) provided the product as a white solid (42.3 mg, 23.7 μmol) in 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, 2H, J = 7.5 Hz), 7.59 (d, 2H, J = 7.3 Hz), 7.14-7.42 (m, 6H), 6.95 (bs, 1H), 6.75 (bd, 1H), 6.55 (d, 1H, J = 9.4 Hz), 5.32 (d, 1H, J = 9.5 Hz), 6.10 (bs, 1H), 5.39 (m, 3H), 5.15-5.28 (m, 3H), 4.84-4.92 (m, 3H), 3.39-4.59 (m, 28H), 2.18 (s, 3H), 2.16 (s, 3H), 2.15 (s, 3H), 2.05 (s, 3H), 2.04 (s, 6H), 2.03 (s, 3H), 2.01 (s, 3H), 2.00 (s, 6H), 1.99 (s, 3H), 1.96 (s, 3H), 1.25-1.95 (m, 18H), 1.45 (s, 9H); MS (LR-ESI) calculated for $C_{83}H_{116}N_8O_{35}Na$ $[M+Na]^+$ 1807.8, found 1807.8.



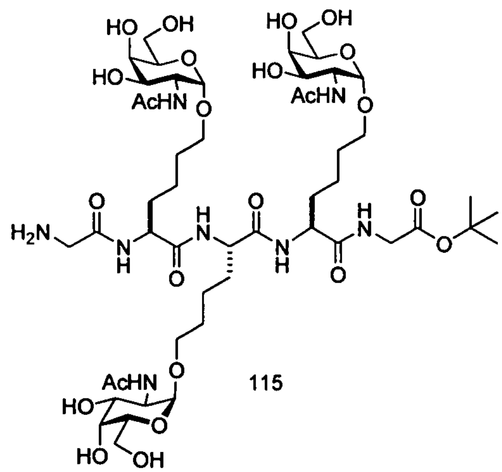
113

[0361] Fmoc-Gly-Nle(α-Tn)-Nle(α-Tn)-Nle(α-Tn)-Gly-OH **113** (Figure 27). Pentapeptide **112** (25.6 mg, 14.3 μmol) was treated with 2.0 mL 95% TFA (aq) at room temperature for 90 minutes. The reaction was concentrated in *vacuo* and triturated with Et_2O 3x to provide the product as a white solid in quantitative yield. HPLC retention time = 28.9 min (gradient 5 to 85% B over 40 min.); MS (LR-ESI) calculated for $C_{79}H_{108}$ $N_8O_{35}Na$ $[M+Na]^+$ 1751.7, found 1751.8.



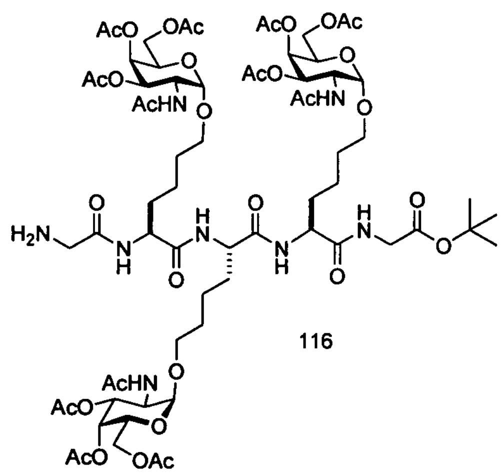
114

[0362] Cbz-Gly-Nle(α-Tn)-Nle(α-Tn)-Nle(α-Tn)-Gly-OH **114 (carbohydrate sectors deprotected)** – See Figure 27. Pentapeptide **111** (14.3 mg, 8.42 μmol) was treated with 2.0 mL 95% TFA (aq) at room temperature for 90 minutes. The reaction was concentrated in *vacuo* and triturated with Et_2O 3x to provide the product as a white solid in quantitative yield. To a solution of the peracylated pentapeptide acid dissolved in MeOH (3 mL) at 0 °C was added dropwise 0.1N NaOH (0.83 mL, 83.4 μmol). The reaction was stirred at 0 °C for 12 hours, neutralized to pH 4-5 with Amberlyst resin, filtered and concentrated in vacuo. Purification via P2-BioGel (BioRad) with water eluent provided the desired compound. HPLC retention time = 13.6 min (gradient 5 to 55% B over 25 min.); MS (LR-ESI) calculated for $C_{54}H_{86}$ $N_8O_{26}Na$ $[M+Na]^+$ 1285.6, found 1285.7.



115

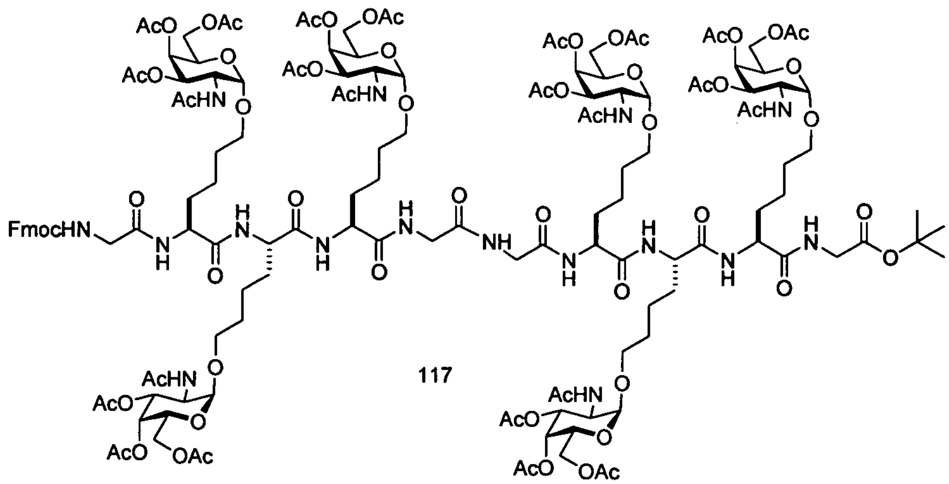
[0363] H-Gly-Nle(α-Tn)-Nle(α-Tn)-Nle(α-Tn)-Gly-OtBu **115 (carbohydrate sectors deprotected)** – See Figure 27. Pentapeptide **112** (19.7 mg, 11.0 μmol) was deprotected according to General Procedure B. Purification (5 to 25% MeOH in CH_2Cl_2) provided the product as a white solid (13.3 mg, 8.51 μmol) in 77% yield. To a solution of the peracylated pentapeptide amine (7.1 mg, 4.54 μmol) dissolved in MeOH (0.9 mL) at 0 °C was added dropwise 0.1N NaOH (0.45 mL, 45.0 μmol). The reaction was stirred at 0 °C for 12 hours, neutralized to pH 4-5 with Amberlyst resin, filtered and concentrated in vacuo. Purification via P2-BioGel (BioRad) with water eluent provided the desired compound. HPLC retention time = 12.2 min (gradient 5 to 55% B over 25 min.); MS (LR-ESI) calculated for $C_{50}H_{89}N_8O_{24}$ $[M+H]^+$ 1185.6, found 1185.7.



116

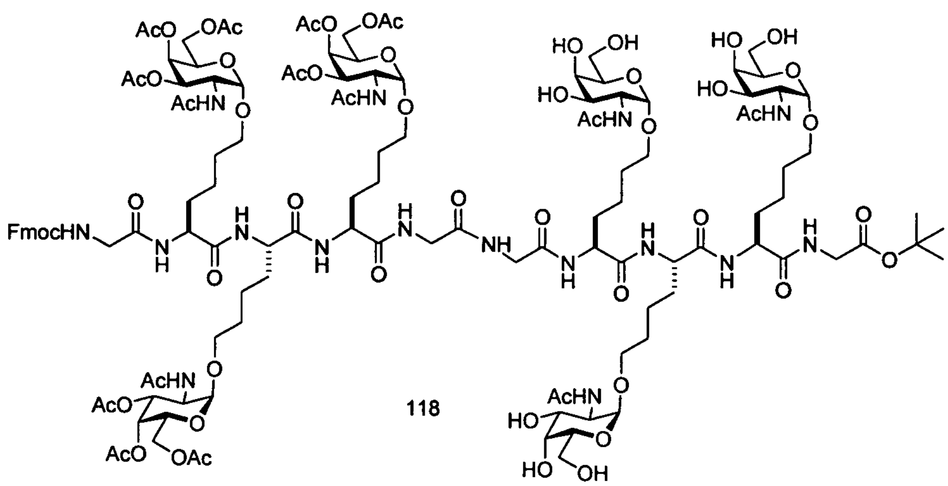
[0364] H-Gly-Nle(α-Tn)-Nle(α-Tn)-Nle(α-Tn)-Gly-OtBu **116** (Figure 27). Pentapeptide **112** (19.7 mg, 11.0 μmol) was deprotected according to General Procedure B.

Purification (5 to 25% MeOH in CH_2Cl_2) provided the product as a white solid (13.3 mg, 8.51 μmol) in 77% yield. MS (LR-ESI) calculated for $C_{68}H_{107}N_8O_{33}$ $[M+H]^+$ 1563.7, found 1563.8.



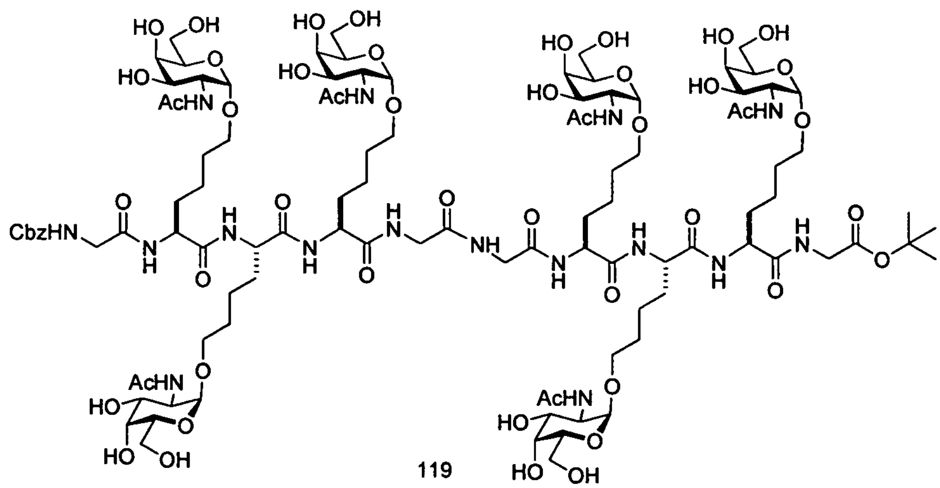
117

[0365] **Compound 117** (Protected Tn plus Protected Tn) – See Figure 28. Acid **113** (4.5 mg, 2.60 μmol) was reacted with amine **116** (3.9 mg, 2.48 μmol) according to General Procedure A. Purification (2 to 9% MeOH in CH_2Cl_2) provided the product as a white solid. MS (LR-ESI) calculated for $C_{147}H_{212}N_{16}O_{67}Na$ $[M+Na]^+$ 3296.4, found 3296.5.



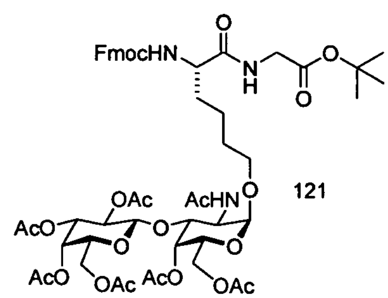
118

[0366] **Compound 118** (Protected Tn plus Deprotected Tn) – See Figure 29. To a solution of Acid **7** (2.3 mg, 1.35 μmol) in 0.25 mL NMP was added DIEA (0.9 μL, 5.4 μmol), HOAT (0.2 mg 1.35 μmol), and HATU (1.0 mg, 2.70 μmol). The solution was stirred for 30 min and amine **115** (1.6 mg, 1.35 μmol) was added. The reaction was stirred for 12 hours. C18 RP-HPLC retention time = 26.7 min (gradient 5 to 85% B over 40 min.); MS (LR-ESI) calculated for $C_{129}H_{195}N_{16}O_{58}$ $[M+H]^+$ 2896.3, found 2896.1.



119

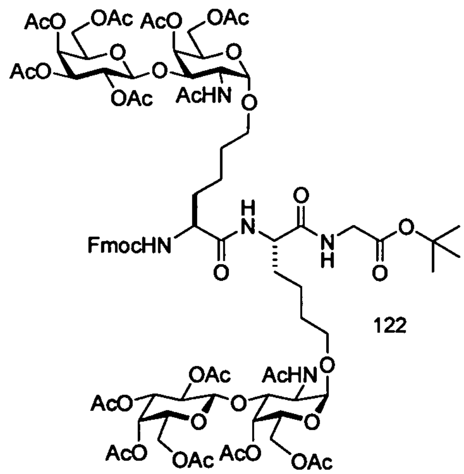
[0367] **Compound 119** (Deprotected Tn plus Deprotected Tn) – See Figure 30. To a solution of Acid **114** (0.53 mg, 0.422 μmol) in 1.0 mL NMP was added DCC (0.3 mg 1.27 μmol) and HOBt (0.2 mg, 1.27 μmol). The solution was stirred for 2 hours and amine **115** (0.5 mg, 0.422 μmol) was added. The reaction was stirred for 12 hours. C18 RP-HPLC retention time = 15.1 min (gradient 5 to 55% B over 25 min.); MS (LR-ESI) calculated for $C_{104}H_{173}N_{16}O_{49}$ $[M+H]^+$ 2430.1, found 2430.1.



121

[0368] Fmoc-Nle(α-TF)-Gly-OtBu **121** (Figure 31). Acid **120** (50 mg, 50.7 μmol) was reacted with Gly-OtBu according to General Procedure A. Purification (0 to 3% MeOH in CH_2Cl_2) provided the product as a white solid in quantitative yield. ^1H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, 2H, J = 7.5 Hz), 7.57-7.61 (m, 2H), 7.40 (t, 2H, J = 7.4 Hz), 7.31 (t, 2H, J = 7.5 Hz), 6.78 (bs, 1H), 6.26 (d, 1H, J = 8.4 Hz), 5.67 (d, 1H, J = 7.7 Hz), 5.36 (d, 1H, J = 3.1 Hz), 5.33 (d, 1H, J = 3.2 Hz), 5.10 (dd, 1H, J = 10.4, 7.9 Hz), 4.96 (dd, 1H, J = 10.4, 3.4 Hz), 4.86 (d, 1H, J = 3.6 Hz), 4.71 (d, 1H, J = 7.7 Hz), 3.86-4.52 (m, 14H), 3.69 (m, 1H), 3.44 (m, 1H), 2.16 (s, 3H), 2.14 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.25-1.80 (m, 6H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 171.0, 170.7, 170.5, 170.4, 170.3, 170.2, 169.8, 168.7, 162.7, 143.9, 141.5, 128.0, 127.2, 125.1, 120.2, 100.9, 98.0, 82.7, 72.8, 71.0,

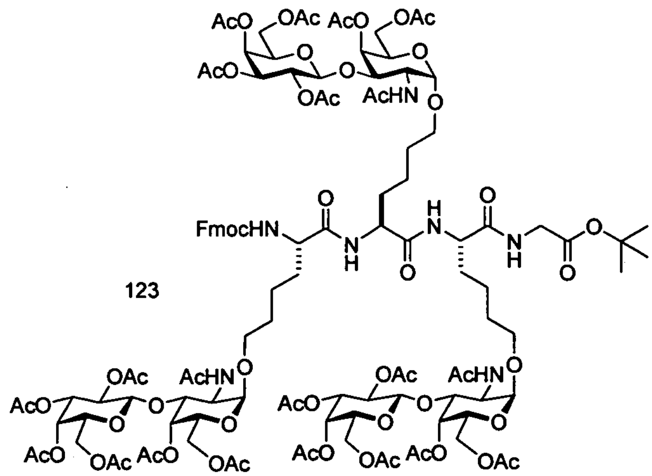
70.7, 69.2, 68.8, 68.4, 67.3, 67.2, 67.0, 62.9, 61.2, 54.9, 53.6, 49.2, 47.3, 42.2, 36.6, 32.2, 31.6, 28.2, 23.4, 23.0, 21.0, 20.9, 20.8, 20.7; MS (LR-ESI) calculated for $C_{53}H_{69}N_3O_{22}Na$ $[M+Na]^+$ 1122.4, found 1122.5.



122

[0369] Fmoc-Nle(α-TF)-Nle(α-TF)-Gly-OtBu **122** (Figure 31). Dipeptide **121** (50.7 μmol) was deprotected according to General Procedure B. Purification (5 to 20% MeOH in CH_2Cl_2) provided the product as a white solid (37.6 mg, 42.8 μmol) in 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (t, 1H, J = 5.3 Hz), 6.68 (d, 1H, J = 9.2 Hz), 5.36 (m, 2H), 5.11 (dd, 1H, J = 10.5, 7.9 Hz), 4.98 (dd, 1H, J = 10.5, 3.5 Hz), 4.85 (d, 1H, J = 3.5 Hz), 4.78 (d, 1H, J = 7.8 Hz), 4.52 (m, 1H), 3.90-4.17 (m, 9H), 3.69 (m, 1H), 3.44-3.48 (m, 2H), 2.17 (s, 3H), 2.13 (s, 3H), 2.07 (s, 6H), 2.04 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.25-1.90 (m, 6H), 1.45 (s, 9H). MS (LR-ESI) calculated for $C_{38}H_{59}N_3O_{20}Na$ $[M+Na]^+$ 900.4, found 900.3. The free amine (37.6 mg, 42.8 μmol) was reacted with acid **120** (44 mg, 44.9 μmol) according to General Procedure A. Purification (1 to 5% MeOH in CH_2Cl_2) provided the product as a white solid (74.1 mg, 40.1 μmol) in 94% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, 2H, J = 7.5 Hz), 7.59-7.65 (m, 2H), 7.41 (t, 2H, J = 7.4 Hz), 7.33 (t, 2H, J = 7.4 Hz), 6.97 (m, 1H), 6.40 (d, 1H, J = 9.0 Hz), 6.25 (d, 1H, J = 8.6 Hz), 5.93 (d, 1H, J = 8.2 Hz), 3.81-5.40 (m, 34H), 3.56-3.70 (m, 2H), 3.33-3.46 (m, 2H), 2.17 (s, 6H), 2.16 (s, 6H), 2.14 (s, 6H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 9H), 1.98 (s, 3H), 1.97 (s, 3H), 1.25-1.90 (m, 12H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 171.4, 171.3, 171.1, 170.0, 170.8, 170.7, 170.6, 170.5, 170.4, 170.3, 170.3, 170.2, 170.2, 170.1, 169.9, 169.8, 169.7, 168.9, 168.7, 143.7, 141.4, 128.1, 127.3, 125.1, 120.3, 102.2, 100.8, 97.8, 97.7, 82.6, 73.2, 73.1, 71.0, 70.9, 70.7, 70.6, 70.5, 69.7, 69.6, 69.3, 69.0, 68.8, 68.0, 67.5,

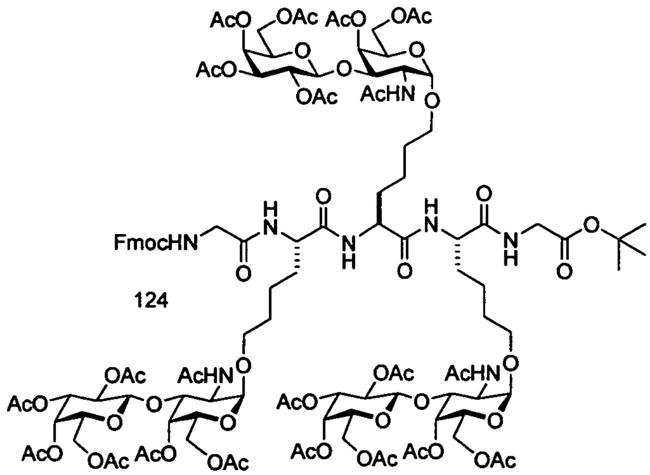
67.3, 67.2, 66.9, 66.8, 62.9, 61.2, 61.1, 55.3, 55.0, 53.2, 53.0, 49.3, 49.1, 47.1, 42.1, 32.0, 31.9, 31.6, 28.4, 28.2, 23.3, 22.7, 22.4, 21.0, 20.9, 20.8, 20.7, 20.7; MS (LR-ESI) calculated for $C_{85}H_{115}N_5O_{40}Na$ $[M+Na]^+$ 1868.7, found 1868.8.



123

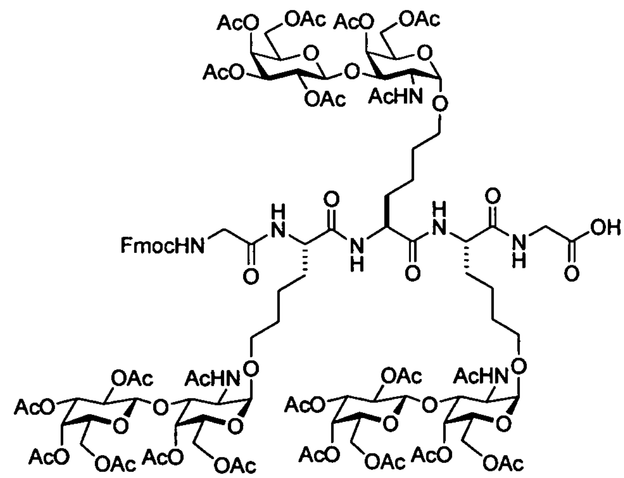
[0370] Fmoc-Nle(α-TF)-Nle(α-TF)-Nle(α-TF)-Gly-OtBu **123** (Figure 31). Tripeptide **122** (74.1 mg, 40.1 μmol) was deprotected according to General Procedure B. Purification (5 to 20% MeOH in CH_2Cl_2) provided the product as a white solid (59.1 mg, 36.4 μmol) in 91% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, 1H, J = 8.0 Hz), 6.95 (t, 1H, J = 5.1 Hz), 6.63 (d, 1H, J = 9.0 Hz), 6.26 (d, 1H, J = 9.1 Hz), 5.35 (m, 5H), 5.09-5.18 (m, 3H), 5.03 (dd, 1H, J = 10.5, 3.3 Hz), 4.97 (dd, 1H, J = 10.5, 3.4 Hz), 4.68-4.87 (m, 5H), 4.42-4.56 (m, 3H), 3.79-4.22 (m, 22H), 3.66-3.73 (m, 2H), 3.39-3.47 (m, 2H), 2.18 (s, 3H), 2.17 (s, 3H), 2.14 (s, 6H), 2.07 (s, 9H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.30-1.90 (m, 12H), 1.46 (s, 9H); MS (LR-ESI) calculated for $C_{70}H_{105}$ $N_5O_{38}Na$ $[M+Na]^+$ 1646.6, found 1646.7. The free amine (59.1 mg, 36.4 μmol) was reacted with acid **120** (37.7 mg, 38.2 μmol) according to General Procedure A. Purification (2 to 7% MeOH in CH_2Cl_2) provided the product as a white solid (83.4 mg, 32.2 μmol) in 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, 2H, J = 7.5 Hz), 7.60 (d, 2H, J = 7.3 Hz), 7.42 (t, 2H, J = 7.3 Hz), 7.33 (t, 2H, J = 7.2 Hz), 6.96-7.20 (m, 2H), 5.93-6.57 (m, 5H), 3.81-5.48 (m, 46H), 3.58-3.69 (m, 3H), 3.39-3.45 (m, 3H), 2.16 (s, 9H), 2.14 (s, 9H), 2.06 (s, 15H), 2.04 (s, 9H), 2.00 (s, 6H), 1.98 (s, 9H), 1.97 (s, 6H), 1.42-1.90 (m, 18H), 1.46 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 171.0, 170.7, 170.6, 170.4, 170.3, 170.2, 169.9, 169.8, 168.6, 143.7, 141.4, 128.1, 127.3, 125.1, 120.3, 100.7, 97.8, 97.7, 82.5, 73.0, 70.9, 70.6, 69.0, 68.8, 68.1, 67.8, 67.5, 67.2, 66.9, 62.8, 61.1, 53.2, 49.2, 47.1,

42.1, 32.0, 28.5, 28.3, 28.1, 23.3, 22.6, 20.9, 20.8, 20.8, 20.7; MS (LR-ESI) calculated for $C_{117}H_{161}N_7O_{58}Na$ $[M+Na]^+$ 2615.0, found 2615.1.



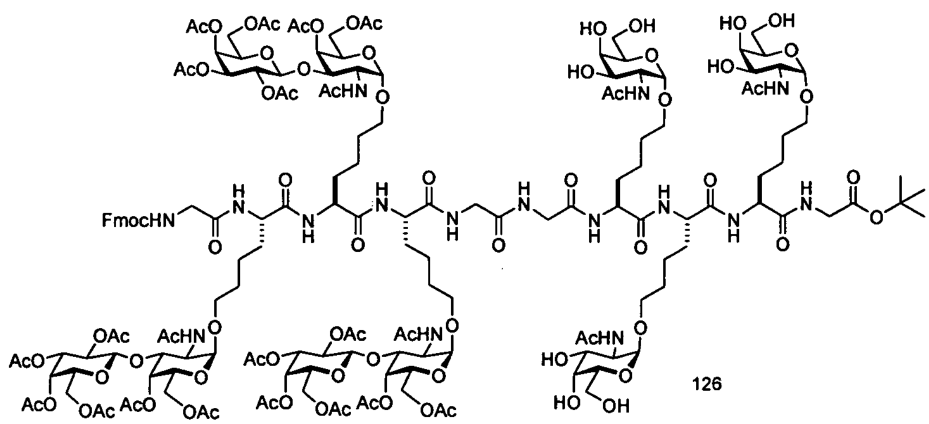
124

[0371] Fmoc-Gly-Nle(α-Tn)-Nle(α-Tn)-Nle(α-Tn)-Gly-OtBu **124** (Figure 31). Tetrapeptide **123** (83.4 mg, 32.2 μmol) was deprotected according to General Procedure B. Purification (5 to 20% MeOH in CH_2Cl_2) provided the product as a white solid (69.9 mg, 29.5 μmol) in 92% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, 1H, J = 7.4 Hz), 7.06 (d, 1H, J = 7.9 Hz), 6.96 (t, 1H, J = 4.9 Hz), 6.54 (d, 1H, J = 8.9 Hz), 6.26 (d, 1H, J = 8.9 Hz), 6.22 (d, 1H, J = 8.8 Hz), 5.33-5.48 (m, 6H), 3.43-5.12 (m, 45H), 2.17 (s, 9H), 2.16 (s, 9H), 2.07 (s, 9H), 2.06 (s, 9H), 2.01 (s, 6H), 1.99 (s, 6H), 1.98 (s, 6H), 1.97 (s, 6H), 1.96 (s, 3H), 1.25-1.90 (m, 18H), 1.47 (s, 9H); MS (LR-ESI) calculated for $C_{102}H_{151}N_7O_{56}Na$ $[M+Na]^+$ 2392.9, found 2393.0. The free amine (69.9 mg, 29.5 μmol) was reacted with Fmoc-Gly-OH according to General Procedure A. Purification (2 to 8% MeOH in CH_2Cl_2) provided the product as a white solid in 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.20-7.79 (m, 16H), 3.41-5.52 (m, 60H), 1.96-2.16 (m, 63H), 1.25-1.95 (m, 18H), 1.45 (s, 9H); MS (LR-ESI) calculated for $C_{119}H_{165}N_8O_{59}Na$ $[M+H]^+$ 2650.0, found 2649.5.



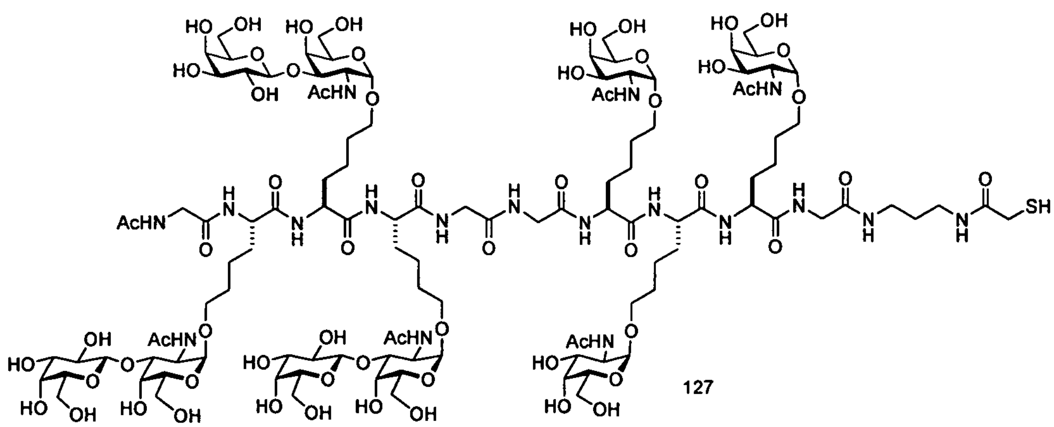
125

[0372] Fmoc-Gly-Nle(α-TF)-Nle(α-TF)-Nle(α-TF)-Gly-OH **125** (Figure 32). Pentapeptide **124** (25.0 mg, 9.43 μmol) was dissolved in 1.0 mL CH_2Cl_2 and treated with 1.0 mL TFA (aq) at room temperature for 2 hours. The reaction was concentrated in *vacuo* and triturated with Et_2O 3x to provide the product as a white solid in quantitative yield. C18 RP-HPLC retention time = 32.1 min (gradient 5 to 85% B over 40 min.); MS (LR-ESI) calculated for $C_{115}H_{157}N_8O_{59}$ $[M+H]^+$ 2593.5, found 2593.7.



126

[0373] **Compound 126** (Protected TF plus Deprotected Tn) – See Figure 33. To a solution of Acid **124** (9.6 mg, 3.71 μmol) in 0.40 mL NMP was added DIEA (1.0 μL), HOAT (0.5 mg 3.37 μmol), and HATU (2.6 mg, 6.74 μmol). The solution was cooled to 0 °C and stirred for 30 min, then amine **115** (4.0 mg, 3.37 μmol) was added. The reaction was stirred for 36 hours. C18 RP-HPLC retention time = 27.8 min (gradient 5 to 85% B over 40 min.); MS (LR-ESI) calculated for $C_{165}H_{242}N_{16}O_{82}$ $[M+2H]^{++}$ 1880.8, found 1880.6.



127

[0374] **Compound 127** (Figure 34). The Fmoc group of **126** will be removed according to General Procedure B and the free amine acetylated by reaction with 1:1 pyridine:acetic anhydride (1 mL). After purification via HPLC the t-butyl ester will be cleaved by treatment with 1:1 CH_2Cl_2:TFA (1.0 mL). The reagents will be removed in vacuo and the residue triturated with Et2O 3x to provide a white solid. The white solid will be dissolved in 1:1 DMF:CH_2Cl_2. HOBt (1.5 eq.) and EDCI (1.5 eq.) will be added followed by HCl-H_2N-$(CH_2)_3$-NH-C(=O)-CH_2-SAc (1.5 eq.) and TEA (1.5 eq.). The reaction will be stirred at room temperature until the starting material has disappeared. The reaction will be concentrated in vacuo and purified via HPLC. The product will then be dissolved in MeOH, cooled to 0 °C and treated with 0.1N NaOH (1.1 eq per acetate to be removed). The reaction will be neutralized with amberlyst resin and purified via P2-BioGel (BioRad) with water eluent.

[0375] *G. Example 7: Immunological Studies*

[0376] It will be appreciated that the inventive glycoconjugates and glycopeptides, as provided herein, are useful for the treatment of cancer and are useful for inducing an antibody response in a subject. Typical protocols for the use of such glycoconjugates and glycopeptides are described in more detail below, and are also detailed in certain references incorporated herein.

[0377] ***1) Preparation of Vaccine***

[0378] Conjugate vaccines **63** and **64** (Figure 23) were prepared as follows. KLH (Sigma Chemical Co, MO, MW 8.6 x 10^6) was modified using *m*-malemidobenzoyl-*N*-hydroxysuccinimide ester (MBS) (Pierce Co., Rockford, IL) as described (Zhang, S., Graeber, L. A., Helling, F., Ragupathi, G., Adluri, S., Lloyd, K. O. & Livingston, P. O. (1996) *Cancer*

Research **56**, 3315-3319). Construct **61**, containing TF, Le^y, and Tn, and construct **62**, containing Globo-H, Le^y, and Tn, were prepared by total synthesis using solution phase peptide synthesis from the appropriately protected constituent glycoamino acids (Williams, L. J., Harris, C. R., Glunz, P. W. & Danishefsky, S. J. (2000) *Tetrahedron Lett.* **41**, 9505-9508. Allen, J. R., Harris, C. A. & Danishefsky, S. J. (2001) *J. Am. Chem. Soc.* **123**, 1890-1897). The sulfhydryl function was incorporated to facilitate attachment to KLH. Global deprotection of the synthetic material revealed compounds **61** and **62**. Addition of construct **61** or **62** to the maleimide-derivatized KLH was achieved by incubating the mixture at room temperature for 3 hours, followed by removal of the unreacted synthetic glycopeptide peptide using a 30,000 molecular cut-off Centriprep filter (Zhang, S., Graeber, L. A., Helling, F., Ragupathi, G., Adluri, S., Lloyd, K. O. & Livingston, P. O. (1996) *Cancer Research* **56**, 3315-3319).

[0379] ***2) Immunization of Mice***

[0380] Groups of five mice (C57BL; Female, 6 weeks of age, from Jackson Laboratory, Bar Harbor, ME) are immunized subcutaneously as follows: Group 1 – immunized with construct **62** (10 μg), plus QS-21(10 μg) (Antigenic Inc., NY, NY); Group 2 – immunized with **62** (10 μg), plus KLH (not conjugated to **62**), plus QS-21 (10 μg); Group 3 – immunized with **4** (3 μg), plus QS-21 (10 μg); Group 4 – immunized with **64** (3 μg), plus GPI-0100 (100 μg) (Galenica Pharmaceuticals Inc., Birmingham, AL); Group 5 – immunized with a mixture containing **104**, **105** and **106** (3 μg each), plus QS-21 (10 μg). Mice were immunized on days 1, 7, and 14, and bled 10 days after third vaccination. The presence of antibody is assayed by an enzyme linked immunosorbent assay (ELISA) as described previously (Ragupathi *et al. Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 125) using the appropriate target antigen (*e.g.*, globo H-ceramide, Le^y ceramide and/or Tn(c)-pamcys as target antigen). The cell surface reactivity can then be tested, for example, the cell surface reactivity of anti- globo H, Le^y, and Tn antibodies are tested on globo H, Le^y, Tn positive cell-lines by flow cytometry assays (FACS).

[0381] **3) Serological Analyses**

[0382] ***Enzyme-Linked Immunosorbent Assay (ELISA).*** ELISAs were performed as previously described (Ragupathi, G., Park, T. K., Zhang, S., Kim, I. J., Graber, L., Adluri, S., Lloyd, K. O., Danishefsky, S. J. & Livingston, P. O. (1997) *Angew. Chem. Int. Ed. Engl.* **36**, 125-128). Briefly, ELISA plates were coated with either synthetic Globo-H–ceramide, or Le^y

and Le^b expressing mucin purified from ovarian cyst fluid (Lloyd, K. O., Kabat, E. A., Layug, E. J. & Gruezo, F. (1966) *Biochemistry* **5**, 1489-1501), or Tn–HAS, or Globo-H–Le^y–Tn–HSA in 0.1 M carbonate buffer (pH 11), at 0.3 μg/well for glycolipids and 0.2 μg/well for glycoproteins. Serially diluted antiserum was added to each well, and alkaline phosphatase-conjugated goat anti-mouse IgM or anti-mouse IgG was added at a dilution of 1:200 (Southern Biotechnology Associates, Inc, Birmingham, AL). Absorbance was measured at 414 nm. ELISA titers are defined as the highest dilution yielding an absorbance of 0.1 or greater over that of normal control mouse sera.

[0383] ***4) Cell Surface Reactivity Determined by FACS.***

[0384] The cell surface reactivity of immune sera was tested on human cell lines as previously described (Ragupathi, G., Park, T. K., Zhang, S., Kim, I. J., Graber, L., Adluri, S., Lloyd, K. O., Danishefsky, S. J. & Livingston, P. O. (1997) *Angew. Chem. Int. Ed. Engl.* **36**, 125-128). Briefly, reactivity was assessed using anti-Globo-H, anti-Le^y, and anti-Tn antibodies tested on MCF-7 (Globo-H and Le^y positive) and LS-C (Tn and Le^y positive) cells (provided by Dr. S. H. Itzkowitz, (Mt. Sinai Hospital, NY)) (Ogata, S., Chen, A. & Itzkowitz, S. H. (1994) *Cancer Res.* **54**, 4036-4044). Single cell suspensions of 2×10^5 cells/tube were washed in PBS with 3% fetal calf serum (FCS) and 0.01M NaN_3 and incubated with 20 μl of 1:20 diluted antisera or monoclonal antibodies for 30 min on ice. The positive control mAbs were VK-9 against synthetic Globo-H (Kudryashov, V., Ragupathi, G., Kim, I. J., Breimer, M. E., Danishefsky, S. J., Livingston, P. O. & Lloyd, K. O. (1998) *Glycoconjugate Journal* **15**, 243-249), 3S193 against Le^y (Hellstrom, I., Garrigues, H. J., Garrigues, U. & Hellstrom, K. E. (1990) *Cancer Res.* **50**, 2183-2190), and αTn against Tn (DAKO Corporation, Carpinteria, CA). After two washes with 3% FCS in PBS, 20 μl of 1:15 diluted goat anti-mouse IgM or IgG-labeled with fluorescein-thiocyanate (FITC) was added, and the mixture incubated for 30 min. After a final wash, the positive population and mean fluorescence intensity of stained cells were differentiated using FACScan (Becton & Dickinson, CA).

[0385] ***5) Discussion of Immunological Studies***

[0386] As discussed above, in one aspect of the invention, multiantigenic glycopeptides are provided. In certain exemplary embodiments, the inventive multiantigenic glycopeptides are trimeric constructs and comprise three different carbohydrate domains found on tumor cell

surfaces. In one embodiment, as shown on Figure 23, the inventive glycopeptides comprise a TF, a Tn and an Le^y epitope (construct **61**). In another embodiment, the inventive glycopeptides comprise a Globo-H, a Tn and an Le^y epitope (construct **63**).

[0387] In the study described herein, the immunological properties of **64** when administered in the presence of QS-21 (Kensil, C. R., Patel, U., Lennick, M. & Marciani, D. (1991) *J. Immunol.* **146**, 431-437) were examined, or, for comparison purposes, when administered in the presense of the related, but less toxic adjuvant GPI-0100 (Marciani, D. J., Press, J. B., Reynolds, R. C., Pathak, A. K., Pathak, V., Gundy, L. E., farmer, J. T., Koratich, M. S. & May, R. D. (2000) *Vaccine* **18**, 3141-3151). As controls, the immunogenicity of the non-conjugated compound **62** was evaluated, and a concurrent investigation on a mixture containing each of the monomeric antigens conjugated to KLH (**104**, **105** and **106**, Figure 25) was conducted. The later experiment was conducted for the purposes of comparing the response by a *single* mouse to *each* of the individual antigens, in response to vaccination with the polyvalent construct versus the mixture of monomers. In order to address issues relating to vaccine formulation, the level of immune response directed against each antigen, within the construct was determined. Furthermore, in anticipation of clinical trials, the ability of antibodies, so generated, to react in vitro with human cell lines known to express the individual antigens was assessed.

[0388] In certain other embodiments, the inventive glycopeptides are conjugated to a suitable carrier either directly, or indirectly through a linker or linker-crosslinker moiety. As discussed above, the carrier may be a protein, polypeptide or a lipid. In certain exemplary embodiments, the inventive glycopeptides are conjugated to the highly immunogenic protein carrier KLH. Vaccine candidates **63** and **64** were prepared by conjugating the totally synthetic glycopeptides **61** and **62**, respectively, to the highly immunogenic protein carrier KLH. QS-21, or the related compound GPI-0100, was used as an adjuvant in order to enhance the immune response against the tumor-associated antigens present in the constructs. Initial ELISA investigations (data not shown) of the two potential conjugate vaccines indicated that construct **64** was superior to **63** from an immunological standpoint, and, consequently, construct **64** was thoroughly investigated. Remarkably, antibodies raised in response to **64** were not only able to identify the individual antigens in ELISAs but, as determined by FACS analysis, they also reacted strongly with tumor cells known to selectively express each tumor-associated antigen.

[0389] Numerous factors could influence the magnitude of the antibody response against individual antigens when more than one antigen is administered during vaccination. The combination of separate pathogen vaccines, such as diphtheria, tetanus, acellular pertussis, hepatitis A, haemophilus influenzae, type b-tetanus toxoid conjugate, and inactivated poliomyelitis, generally results in similar antibody responses against the individual components, whether they are administered separately, simultaneously, or sequentially (Jones, I. G., Tyrrell, H., Hill, A., Horobin, J. M. & Taylors, B. (1988) *Vaccine* **16**, 113; Usonis, V., Bakasenas, V., Williams, P. & Clemens, R. (2000) *Vaccine* **18**, 947-954; and Kanra, G., Silier, T., Yurdakok, K., Yavuz, T., Baskan, S., Ulukol, B., Ceyhan, M., Ozmert, E., Turday, F. & Pehlivan, T. (1999) *Vaccine* **18**, 947-954). Similarly, combining purified bacterial capsular polysaccharides does not seem to reduce the immunogenicity of the individual polysaccharide components. By contrast, for conjugate vaccines, several factors could potentially negatively impact the antibody response to individual antigens, especially when monomeric conjugate vaccines are combined and administered using a cocktail-like approach (See, for example, Anderson, P. (1983) *Infection & Immunity* **39**, 233-238; Kurika, S. (1996) *Vaccine* **14**, 1239-1242; Barington, T., Gyhrs, A., Kristensen, K. & Heilmann, C. (1994) *Infection & Immunity* **62**, 9-14; Peters, C. C., Tenbergen-Meeks, A. M., Poolman, J. T., Beurret, M., Zegers, B. J. M. & Rijkers, G. T. (1974) *Infect. Immun.* **59**, 3504-3510; Sarvas, H., Makela, O., Toivanen, P. & Toivanen, A. (1974) *Scand. J. Immunol.* **3**, 455-460; Fattom, A., Cho, Y. H., Chu, C., Fuller, S., Fries, L. & Naso, R. (1999) *Vaccine* **17**, 126-133; Barington, T., Skettrup, M., Juul, L. & Heilman, C. (1993) *Infect. Immun.* **61**, 432-438; Cross, A. M., Artenstein, A., Qu, J. & *et al.* (1994) *Journal of Infectious Diseases* **170**, 834-840; Sarnaik, S., Kaplan, J., Schiffman, G., Bryla, D., Robbins, J. B. & Schneerson, R. (1990) *Pediatric Infectious Disease* **9**, 181-186; and Molrine, D. C., Tarbell, G. S. & *et al.* (1995) *Annals of International Medicine* **123**, 828-834).

[0390] In some instances, exposure to a carrier appears to produce an amplified response upon subsequent challenge, thereby resulting in increased antibody production against antigens conjugated to the same carrier protein (see, Anderson, P. (1983) *Infection & Immunity* **39**, 233-238; Kurika, S. (1996) *Vaccine* **14**, 1239-1242). In other cases, prior exposure to a carrier results in increased antibody levels only against the carrier and not to the conjugated antigens (See, Barington, T., Gyhrs, A., Kristensen, K. & Heilmann, C. (1994) *Infection & Immunity* **62**, 9-14;

Peters, C. C., Tenbergen-Meeks, A. M., Poolman, J. T., Beurret, M., Zegers, B. J. M. & Rijkers, G. T. (1974) *Infect. Immun.* **59**, 3504-3510; Sarvas, H., Makela, O., Toivanen, P. & Toivanen, A. (1974) *Scand. J. Immunol.* **3**, 455-460). Combining conjugate vaccines containing the same carrier, or simultaneous administration of the carrier in non-conjugated form, may result in a decreased antibody response against the target antigens (See, Fattom, A., Cho, Y. H., Chu, C., Fuller, S., Fries, L. & Naso, R. (1999) *Vaccine* **17**, 126-133; Barington, T., Skettrup, M., Juul, L. & Heilman, C. (1993) *Infect. Immun.* **61**, 432-438; Cross, A. M., Artenstein, A., Qu, J. & *et al.* (1994) *Journal of Infectious Diseases* **170**, 834-840; Sarnaik, S., Kaplan, J., Schiffman, G., Bryla, D., Robbins, J. B. & Schneerson, R. (1990) *Pediatric Infectious Disease* **9**, 181-186; and Molrine, D. C., Tarbell, G. S. & *et al.* (1995) *Annals of International Medicine* **123**, 828-834). Without wishing to be bound to any particular theory, Applicant proposes that these complications could be avoided through combination of the various antigens on the same conjugate vaccine. There was a possibility that, upon combining several antigens within the same construct, the immune response against one or more of the members of that set could be suppressed. Additionally, cross-reactivity involving more than one antigen might be observed, which would be expected to result in a portion of the antibody population produced having reduced affinity to particular antigens displayed on the cell surface. With that in mind, the effect of the administration of a glycopeptide bearing different antigens on the imminus response in subject was investigated. Significantly, the studies with compound **64** revealed that there was no substantial decrease in antibody titers over the course of immunizations with **64**. Furthermore, no indication of an impaired antibody response against the individual antigens within the construct was apparent, as assessed by ELISA and FACS analysis. In fact, the antibody response for each individual antigen within the clustered construct was similar to that observed when the mixture of individual monomers was administered. What is even more significant from a potential therapeutic point of view is that, compared to the mixture of monomers, antibodies raised to the multivalent construct exhibited equal or higher reactivity with human cell lines expressing the native antigens, as determined by FACS analysis. Interestingly, although relatively high ELISA-based antibody titers were observed when sera resulting form vaccination with the polyvalent construct were screened against the polyvalent construct itself (see Table 5), FACS-based analyses (see Table 6) showed that that sera reacted just as well as sera derived form the monomers. The ELISA-based data in this case would seem to suggest that there was,

indeed, cross-reactivity of antibodies between the antigens in the multivalent construct. However, the FACS data clearly indicate that this cross-reactivity does not negatively impact recognition of the antigens on the cell surface.

[0391] *Antibody Response against Globo-H–Le^y–Tn construct (62).* ELISA antibody titers against Globo-H–Le^y–Tn in sera from mice immunized with **64** was determined and results are summarized in Table 5. Relatively strong IgM and IgG titers were detected in mice vaccinated with **64**, compared to pre-vaccination sera which showed no IgG and IgM titers. Construct **64** induced both IgM and IgG antibodies, with the GPI-0100 group inducing significantly higher titers compared to the QS-21 group. Group 5 induced very low IgM and IgG titers against the multivalent construct (see Table 5), when compared with groups 3 and 4.

[0392] *Antibody Response against Globo-H ceramide.* ELISA antibody titers against Globo-H–ceramide in sera from mice immunized with **64** were determined. As summarized in Table 5, weak IgM titers were detected in pre-vaccination sera, while sera obtained after vaccination with **64** showed increased IgM and IgG titers. No difference in IgM titers between groups 3 and 4 was detected. However, the group receiving QS-21 induced IgG antibodies against Globo-H, whereas the group receiving GPI-0100 failed to do so. Sera obtained from group 5 reacted strongly with Globo-H, relative to all other groups.

[0393] *Antibody Response against Le^y.* ELISA antibody titers against Le^y in sera from mice immunized with **64** were tested and the results are summarized in Table 5. With the exception of group 1, no detectable anti-Le^y antibodies were present in pre-vaccination sera. In general, sera obtained following vaccination with **64**, or a mixture of three vaccines, reacted relatively strongly with Le^y by ELISA. Construct **64** induced both IgM and IgG antibodies against Le^y. No difference in antibody production was observed between groups 3 and 4, having received QS-21 and GPI-0100, respectively. No difference in antibody titers was observed between mice immunized with construct **62**, or with a mixture containing monomeric constructs **104**, **105**, and **106**.

[0394] *Antibody Response against Tn antigen.* ELISA antibody titers against Tn–HSA in sera from mice immunized with **64** and mixture of constructs **104**, **105**, and **106** were determined. As summarized in Table 5, no IgM or IgG activity was detected in pre-vaccination sera. Groups 3 and 4 induced both IgM and IgG titers against Tn, but the adjuvant GPI-0100

induced one fold higher titer than adjuvant QS-21. Group 5 also showed high IgG titers against Tn antigen.

[0395] *Cell surface reactivities.* Cell surface reactivity of the sera was tested by flow cytometry using MCF-7 (Globo-H, Le^y and Tn positive) and LS-C (Tn and Le^y positive) cell lines. The results are summarized in Table 6, and the histograms of FACS against MCF-7 for groups 3, 4 and 5 are presented in Figure 25. Sera obtained from all pre-vaccinated mice showed minimal reactivity (<10% positive cells). Following vaccination, groups 3, 4 and 5 showed significant IgM reactivity and low IgG reactivity against MCF-7 cells. No significant difference in cell surface reactivity against MCF-7 was observed with sera obtained after vaccination with construct **64** (group 3) or a mixture of constructs **104**, **105**, and **106** (group 5). There also did not appear to be a difference in cell surface reactivity between the adjuvants QS-21 (group 3) and GPI-0100 (group 5).

[0396] Without wishing to be bound to any particular theory, Applicant proposes that the lack of suppression of the antibody response against these multiantigenic vaccines may be due to the KLH/adjuvant combination (See, for example, Helling, F., Shang, A., Calves, M., Zhang, S., Ren, S., Yu, R. K., Oettgen, H. F. & Livingston, P. O. (1994) *Cancer Research* **54**, 197-203; Kim, S. K., Ragupathi, G., Musselli, C., Choi, S. J., Park, Y. S. & Livingston, P. O. (2000) *Vaccine* **18**, 597-603; and Kim, S., Ragupathi, G., Cappello, S., Kagan, E. & Livingston, P. O. (2000) *Vaccine* **19**, 530-537). The use of KLH as carrier and QS-21 as adjuvant has been shown to result in a potent helper T cell type-1 response (See Helling, F., Shang, A., Calves, M., Zhang, S., Ren, S., Yu, R. K., Oettgen, H. F. & Livingston, P. O. (1994) *Cancer Research* **54**, 197-203). This is likely the case for GPI-0100 as well, given its close structural relationship to QS-21. It has been reported that KLH is more effective as an immunogenic carrier than are a variety of other standard proteins. It has also been demonstrated that for GD3–KLH and MUC1–KLH conjugates, adjuvants such as QS-21 induce a 1000-100,000 fold augmentation of antibody responses in the mouse, compared to the use of the conjugates alone (See Kim, S. K., Ragupathi, G., Musselli, C., Choi, S. J., Park, Y. S. & Livingston, P. O. (2000) *Vaccine* **18**, 597-603; and Kim, S., Ragupathi, G., Cappello, S., Kagan, E. & Livingston, P. O. (2000) *Vaccine* **19**, 530-537). However, since our goal in the present study was primarily that of determining whether a

multivalent conjugate vaccine could be administered without clear loss of immunogenicity against the individual components, we did not attempt to saturate the system.

[0397] Several other observations are noteworthy. Since GPI-0100 is less toxic than QS-21, greater quantities of GPI-0100 could be safely administered to the mice, and this resulted in a commensurate increase in antibody production. Also regarding antibody production, in general, construct **63** produced lower titers than **64**. The structural differences between **63** and **64** may account for the immunological variance observed for these vaccines. Compound **63** is a more accurate mimic of mucin glycoproteins. Clustered glycoamino acids containing the mucin α-*O*-linked GalNAc core are highly rigidified, even in the case of very short glycopeptides, as a result of specific interactions between the glycan and peptide backbone (Don M. Coltart, Ajay K. Royyuru, Lawrence J. Williams, Peter W. Glunz, Dalibor Sames, Scott D. Kuduk, Jacob B. Schwarz, Xiao-Tao Chen, Samuel J. Danishefsky, David H. Live *J. Am. Chem. Soc.* In press). Thus, as a result of such structurally-based interactions, use of a mucin mimic that is faithful to the known architectural features of the cell surface molecule might impede the identification of the individual constituent antigens displayed on the peptide backbone during the immune response. In addition, the close resemblance of the structural core of the mucin-based vaccine construct (**63**) to self antigens within the mice, might make it more difficult to break tolerance.

[0398] In summary, single vaccine constructs bearing several different carbohydrate antigens such as those described herein, have the potential to stimulate a multifaceted immune response, necessary for optimal targeting of the heterogenous population of cells associated with a particular cancer type. Thus, since Globo-H, Le^y, and Tn are each over-expressed on prostate cancer, vaccination with **64** could potentially induce a broader range of antibodies, which will have a greater likelihood of accomplishing immunosurveillance against a greater range of aberrant cells. One of ordinary skill in the art will apreciate that the methods disclosed herein for the preparation of these constructs disclosed herein, as well as methods disclosed elsewhere (see, for example, Williams, L. J., Harris, C. R., Glunz, P. W. & Danishefsky, S. J. (2000) *Tetrahedron Lett.* **41**, 9505-9508; Allen, J. R., Harris, C. A. & Danishefsky, S. J. (2001) *J. Am. Chem. Soc.* **123**, 1890-1897 and U.S. Patent Application Nos.: 09/083,776 and 09/276,595) are readily adaptable to the inclusion of more complex patterns of glycosylation and more elaborate peptide motifs, which might activate other elements of the immune system.

Table 5: Vaccination-induced ELISA-based antibody titers

(Group) Vaccine Formulation	Mouse*	Against Multivalent construct: Pre-vaccination IgM	Pre-vaccination IgG	10 days post 3rd vaccination IgM	10 days post 3rd vaccination IgG	Against Globo-H: Pre-vaccination IgM	Pre-vaccination IgG	10 days post 3rd vaccination IgM	10 days post 3rd vaccination IgG	Against Le^y: Pre-vaccination IgM	Pre-vaccination IgG	10 days post 3rd vaccination IgM	10 days post 3rd vaccination IgG	Against Tn: Pre-vaccination IgM	Pre-vaccination IgG	10 days post 3rd vaccination IgM	10 days post 3rd vaccination IgG
(1)	1.1	0	0	0	0	320	40	0	0	0	80	0	0	0	0	0	0
	1.2	0	0	0	0	640	0	320	0	0	160	0	0	0	0	0	0
10 μg Globo-H–Le^y–	1.3	0	0	0	0	0	0	40	0	0	40	0	0	0	0	0	0
Tn + 10 μg QS-21	1.4	0	0	0	0	40	0	0	40	0	160	0	0	0	0	40	0
	1.5	0	0	0	0	320	160	80	40	0	160	0	0	0	0	80	0
	Median	**0**	**0**	**0**	**0**	**320**	**0**	**40**	**0**	**0**	**160**	**0**	**0**	**0**	**0**	**0**	**0**
(2)	2.1	0	0	320	320	0	0	40	40	0	0	80	40	0	0	80	0
	2.2	0	0	160	640	0	80	320	0	0	0	0	40	0	0	160	320
10 μg Globo-H–Le^y–	2.3	0	0	0	320	0	40	0	0	0	0	0	80	0	0	0	320
Tn + KLH	2.4	0	0	160	320	160	0	320	40	0	0	40	0	0	0	80	160
(nonconjugated) +	2.5	0	0	160	320	80	0	160	40	0	0	80	40	0	0	40	160
10 μg QS-21	Median	**0**	**0**	**160**	**320**	**0**	**0**	**160**	**0**	**0**	**0**	**40**	**40**	**0**	**0**	**80**	**160**
(3)	3.1	0	0	160	5120	160	0	80	0	0	0	40	40	0	0	80	320
	3.2	0	0	640	10240	0	40	0	0	0	0	320	80	0	0	80	0
3 μg Globo-H–Le^y–	3.3	0	0	1280	10240	0	0	80	40	0	0	160	80	0	0	320	80
Tn–KLH +	3.4	0	0	640	2560	0	0	40	40	0	0	40	80	0	0	320	320
10 μg QS-21	3.5	0	0	320	10240	0	0	40	40	0	0	80	80	0	0	160	80
	Median	**0**	**0**	**640**	**10240**	**0**	**0**	**40**	**40**	**0**	**0**	**80**	**80**	**0**	**0**	**160**	**160**
(4)	4.1	0	0	640	40960	80	0	40	0	0	0	80	40	0	0	160	160
	4.2	0	0	160	20480	0	0	160	0	0	0	160	40	0	0	320	160
3 μg Globo-H–Le^y–	4.3	0	0	2560	40960	0	0	0	0	0	0	80	40	0	0	320	640
Tn–KLH +	4.4	0	0	2560	20480	0	0	40	0	0	0	640	320	0	0	320	640
100 μg GPI-0100	4.5	0	0	1280	40960	0	0	0	0	0	0	80	0	0	0	1280	1280
	Median	**0**	**0**	**1280**	**40960**	**0**	**0**	**40**	**0**	**0**	**0**	**80**	**40**	**0**	**0**	**320**	**640**
(5)	5.1	0	0	80	640	0	0	160	40	0	0	40	80	0	0	1280	81920
	5.2	0	0	640	320	0	0	320	160	0	0	160	320	0	0	80	10240
3 μg Globo-H–KLH, 3	5.3	80	0	0	0	0	0	160	40	0	0	160	80	0	0	5120	81920
μg Le^y–KLH, 3 μg Tn–	5.4	0	0	1280	640	160	0	160	0	0	0	80	160	0	0	1280	163840
KLH +	5.4	0	0	2560	1280	160	0	320	80	0	0	160	80	0	0	1280	40960
10 μg QS-21	Median	**0**	**0**	**640**	**640**	**0**	**0**	**160**	**40**	**0**	**0**	**160**	**80**	**0**	**0**	**1280**	**81920**

*Each number corresponds to an individual mouse.

Table 6: FACS assay on MF7 and LSC cell lines with immune sera obtained pre- and post-immunization

(Group) Vaccine Formulation	Mouse*	% Positive cells by FACS (MFI)* MCF-7 human breast cell line Pre-serum IgM	IgG	Post-serum IgM	IgG	LSC human colon cell line Pre-serum IgM	IgG	Post-serum IgM	IgG
(1)	1.1	10 (21)	11 (12)	5 (18)	11 (11)	10 (31)	10 (12)	12 (36)	12 (14)
	1.2	9 (13)	9 (12)	31 (18)	8 (9)	11 (30)	10 (17)	11 (31)	11 (15)
10 μg Globo-H–Le^y–Tn +	1.3	10 (15)	11 (10)	15 (17)	9 (11)	10 (52)	10 (12)	10 (50)	11 (12)
10 μg QS-21	1.4	10 (5)	11 (9)	28 (11)	16 (11)	11 (28)	10 (10)	7 (22)	15 (14)
	1.5	10 (14)	10 (5)	29 (20)	15 (26)	10 (32)	11 (11)	10 (32)	14 (14)
	Median	**10 (14)**	**11 (10)**	**28 (18)**	**11 (11)**	**10 (31)**	**10 (12)**	**10 (32)**	**12 (14)**
(2)	2.1	10 (51)	10 (12)	31 (65)	11 (11)	10 (20)	11 (13)	15 (21)	18 (17)
	2.2	11 (44)	10 (10)	43 (79)	18 (16)	11 (19)	11 (13)	24 (29)	29 (19)
10 μg Globo-H–Le^y–Tn +	2.3	11 (41)	9 (9)	68 (99)	13 (15)	10 (34)	11 (11)	22 (50)	14 (12)
KLH (nonconjugated) +	2.4	11 (38)	10 (7)	82 (127)	20 (16)	11 (54)	11 (17)	12 (61)	23 (37)
10 μg QS-21	2.5	10 (44)	10 (10)	51 (86)	20 (16)	10 (20)	11 (11)	45 (67)	21 (19)
	Median	**11 (44)**	**10 (10)**	**51 (86)**	**18 (16)**	**10 (20)**	**10 (13)**	**22 (50)**	**21 (19)**
(3)	3.1	10 (42)	11 (8)	46 (62)	20 (11)	10 (14)	11 (22)	20 (18)	7 (17)
	3.2	11 (34)	10 (5)	93 (144)	48 (18)	10 (13)	10 (5)	70 (32)	68 (24)
3 μg Globo-H–Le^y–Tn–	3.3	11 (36)	11 (10)	76 (109)	26 (16)	10 (64)	10 (25)	18 (94)	30 (68)
KLH +	3.4	11 (43)	11 (7)	53 (89)	23 (12)	10 (70)	11 (52)	20 (107)	14 (60)
10 μg QS-21	3.5	11 (33)	11 (7)	77 (95)	39 (15)	10 (62)	10 (20)	24 (95)	38 (53)
	Median	**11 (36)**	**11 (7)**	**76 (95)**	**26 (15)**	**10 (62)**	**10 (22)**	**20 (94)**	**30 (53)**
(4)	4.1	11 (33)	10 (8)	47 (65)	17 (10)	11 (13)	10 (8)	23 (18)	25 (13)
	4.2	12 (39)	10 (8)	66 (102)	36 (25)	10 (16)	10 (10)	31 (26)	43 (17)
3 μg Globo-H–Le^y–Tn–	4.3	10 (37)	10 (11)	73 (100)	8 (11)	11 (71)	10 (28)	18 (122)	19 (50)
KLH +	4.4	12 (41)	10 (7)	87 (167)	23 (12)	11 (63)	9 (23)	72 (251)	36 (67)
100 μg GPI-0100	4.5	11 (34)	10 (8)	55 (76)	57 (26)	10 (58)	10 (22)	13 (66)	29 (54)
	Median	**11 (37)**	**10 (8)**	**66 (100)**	**23 (12)**	**11 (58)**	**10 (22)**	**23 (66)**	**29 (50)**
(5)	5.1	12 (47)	11 (10)	41 (69)	14 (11)	10 (14)	10 (13)	14 (17)	16 (24)
	5.2	12 (35)	10 (8)	96 (175)	16 (10)	9 (21)	10 (10)	32 (44)	22 (12)
3 μg Globo-H–KLH, 3 μg	5.3	10 (34)	10 (9)	77 (105)	23 (12)	10 (17)	11 (27)	78 (131)	29 (53)
Le^y–KLH, 3 μg Tn–KLH +	5.4	11 (31)	10 (7)	69 (75)	30 (16)	11 (91)	10 (23)	7 (73)	22 (36)
10 μg QS-21	5.4	12 (17)	9 (7)	96 (89)	25 (11)	10 (69)	10 (27)	18 (110)	52 (70)
	Median	**12 (34)**	**10 (8)**	**77 (89)**	**23 (11)**	**10 (21)**	**10 (23)**	**18 (73)**	**22 (36)**

*Mean fluorescence intensity. Monoclonal antibody 3S193 (IgG) showed 99%, VK-9 showed 42% on MCF-7. 3S193 (IgG) showed 78%, HB-Tn-1 showed 91% on LSC.